WOODHEAD PUBLISHING SERIES IN FOOD SCIENCE, TECHNOLOGY AND NUTRITION



# Improving the safety and quality of milk

## Volume 2: Improving quality in milk products

Edited by Mansel W. Griffiths

CRC

WP

# Improving the safety and quality of milk

© Woodhead Publishing Limited, 2010

## Related titles:

*Dairy processing: improving quality*
(ISBN 978-1-85573-676-4)
With its distinguished international team of contributors, *Dairy processing* summarises key developments in the field and how they enhance dairy product safety and quality. The first part of the book discusses raw milk composition, production and quality. Part II reviews developments in processing from hygiene and HACCP systems to automation, high-pressure processing and modified atmosphere packaging. The final part considers developments for particular products such as fermented dairy products and cheeses.

*Dairy-derived ingredients: food and nutraceutical uses*
(ISBN 978-1-84569-465-4)
Advances in technologies for the extraction and modification of valuable milk components have opened up new opportunities for the food and nutraceutical industries. New applications for dairy ingredients are also being found. *Dairy-derived ingredients* reviews the latest developments in these dynamic areas. The first part covers modern approaches to the separation of dairy components and manufacture of dairy ingredients. The second part focuses on the biological functionality of dairy components and their nutraceutical applications. The final part of the book addresses the technological functionality of dairy components and their applications in food and non-food products.

*Foodborne pathogens: hazards, risk analysis and control Second edition*
(ISBN 978-1-84569-362-6)
Effective control of pathogens continues to be of great importance to the food industry. The first edition of *Foodborne pathogens* quickly established itself as an essential guide for all those involved in the management of microbiological hazards at any stage in the food production chain. This major new edition strengthens that reputation, with extensively revised and expanded coverage, including more than ten new chapters. Part I focuses on risk assessment and management in the food chain. Chapters in this section cover pathogen detection, microbial modelling, the risk assessment procedure, pathogen control in primary production, hygienic design and sanitation, among other topics. Parts II and III then review the management of key bacterial and non-bacterial foodborne pathogens.

Details of these books and a complete list of Woodhead titles can be obtained by:

- visiting our web site at www.woodheadpublishing.com
- contacting Customer Services (email: sales@woodheadpublishing.com; fax: +44 (0) 1223 893694; tel.: +44 (0) 1223 891358 ext. 130; address: Woodhead Publishing Limited, Abington Hall, Granta Park, Great Abington, Cambridge CB21 6AH, UK)

© Woodhead Publishing Limited, 2010

Woodhead Publishing Series in Food Science, Technology and Nutrition:
Number 189

# Improving the safety and quality of milk

## Volume 2: Improving quality in milk products

**Edited by**
**Mansel W. Griffiths**




**CRC Press**
**Boca Raton Boston New York Washington, DC**

WOODHEAD PUBLISHING LIMITED
Oxford Cambridge New Delhi

© Woodhead Publishing Limited, 2010

Published by Woodhead Publishing Limited, Abington Hall, Granta Park,
Great Abington, Cambridge CB21 6AH, UK
www.woodheadpublishing.com

Woodhead Publishing India Private Limited, G-2, Vardaan House, 7/28 Ansari Road,
Daryaganj, New Delhi – 110002, India
www.woodheadpublishingindia.com

Published in North America by CRC Press LLC, 6000 Broken Sound Parkway, NW,
Suite 300, Boca Raton, FL 33487, USA

First published 2010, Woodhead Publishing Limited and CRC Press LLC
© Woodhead Publishing Limited, 2010
The authors have asserted their moral rights.

This book contains information obtained from authentic and highly regarded sources. Reprinted material is quoted with permission, and sources are indicated. Reasonable efforts have been made to publish reliable data and information, but the authors and the publishers cannot assume responsibility for the validity of all materials. Neither the authors nor the publishers, nor anyone else associated with this publication, shall be liable for any loss, damage or liability directly or indirectly caused or alleged to be caused by this book.

Neither this book nor any part may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, microfilming and recording, or by any information storage or retrieval system, without permission in writing from Woodhead Publishing Limited.

The consent of Woodhead Publishing Limited does not extend to copying for general distribution, for promotion, for creating new works, or for resale. Specific permission must be obtained in writing from Woodhead Publishing Limited for such copying.

Trademark notice: Product or corporate names may be trademarks or registered trademarks, and are used only for identification and explanation, without intent to infringe.

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library.

Library of Congress Cataloging in Publication Data
A catalog record for this book is available from the Library of Congress.

Woodhead Publishing ISBN 978-1-84569-806-5 (book)
Woodhead Publishing ISBN 978-1-84569-943-7 (e-book)
CRC Press ISBN 978-1-4398-3639-2
CRC Press order number: N10245

The publishers' policy is to use permanent paper from mills that operate a sustainable forestry policy, and which has been manufactured from pulp which is processed using acid-free and elemental chlorine-free practices. Furthermore, the publishers ensure that the text paper and cover board used have met acceptable environmental accreditation standards.

Typeset by Godiva Publishing Services Limited, Coventry, West Midlands, UK
Printed by TJ International Limited, Padstow, Cornwall, UK

© Woodhead Publishing Limited, 2010

Downloaded From Vet-eBooks.com

# Contents

*Contributor contact details* ..... xi

*Woodhead Publishing Series in Food Science, Technology and Nutrition* ..... xv

*Preface* ..... xxi

**Part I Nutritional aspects of milk**

**1 The role of milk in the diet** ..... 3
*H. Bishop MacDonald, Nutrisphere, Canada*
1.1 Introduction ..... 3
1.2 Milk consumption worldwide ..... 4
1.3 Nutritional benefits of milk ..... 5
1.4 Disadvantages of a low-dairy diet ..... 15
1.5 Sources of further information and advice ..... 19
1.6 References ..... 20

**2 The health aspects of milk** ..... 28
*M. de Vrese, M. Pfeuffer, N. Roos, K. Scholz-Ahrens and J. Schrezenmeir, Max Rubner-Institut (MRI) – Federal Research Institute of Nutrition and Food, Germany*
2.1 Introduction ..... 28
2.2 Bone and teeth health ..... 29
2.3 Hypertension and overall cardiovascular disease (CVD) risk 37

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

2.4 Protection from obesity ..... 44
2.5 Effects of natural and added milk constituents, particularly pro- and prebiotics, on gut health ..... 50
2.6 Sources of further information and advice ..... 60
2.7 References ..... 61

**3 'Designer' milks: functional foods from milk** ..... 74
*M. Boland, Riddet Institute, Massey University, New Zealand*
3.1 Introduction: functional milk components ..... 74
3.2 Milks with manipulated functional properties: production and application ..... 81
3.3 Conclusions and future trends ..... 88
3.4 Sources of further information and advice ..... 89
3.5 Acknowledgements ..... 89
3.6 References ..... 89

**Part II Improving milk quality**

**4 Understanding and preventing consumer milk microbial spoilage and chemical deterioration** ..... 97
*M. Heyndrickx, S. Marchand, V. De Jonghe, K. Smet, K. Coudijzer and J. De Block, Institute for Agricultural and Fisheries Research (ILVO), Belgium*
4.1 Introduction ..... 97
4.2 Spoilage of pasteurised and extended shelf-life (ESL) milk .. 99
4.3 Spoilage of ultra high temperature (UHT) and sterilised milk . 108
4.4 Future trends ..... 122
4.5 Sources of further information and advice ..... 122
4.6 References ..... 123

**5 Effects of packaging on milk quality and safety** ..... 136
*M. Kontominas, University of Ioannina, Greece*
5.1 Introduction ..... 136
5.2 Types of packaging materials and their applications ..... 137
5.3 Factors related to packaging affecting milk shelf-life and safety ..... 145
5.4 Migration and flavor scalping ..... 149
5.5 Environmental issues regarding packaging materials ..... 151
5.6 Sources of further information and advice ..... 155
5.7 References ..... 155

**6 Sensory evaluation of milk** ..... 159
*K. W. Chapman, Cornell University, USA*
6.1 Introduction: key issues in the sensory evaluation of milk ... 159

© Woodhead Publishing Limited, 2010

Downloaded From Vet-eBooks.com

6.2 Historical perspective ..... 160
6.3 Principles of sensory evaluation ..... 161
6.4 Examples of evaluation methods, their application and effectiveness ..... 161
6.5 Application of advanced statistical methods ..... 172
6.6 Sources of further information and advice ..... 174
6.7 References and further reading ..... 177

**7 Instrumental measurement of milk flavour and colour** ..... 181
*K. Cadwallader, University of Illinois, USA*
7.1 Introduction ..... 181
7.2 Flavour and colour of milk ..... 182
7.3 Flavour measurement ..... 188
7.4 Measurement of colour ..... 197
7.5 Future trends ..... 198
7.6 Sources of further information and advice ..... 198
7.7 References ..... 198

**8 Analysing and improving the mineral content of milk** ..... 207
*F. Gaucheron, INRA – Agrocampus Ouest, France*
8.1 The minerals of milk ..... 207
8.2 Methods for analysing the mineral content in milk ..... 210
8.3 Improving the mineral content in milk ..... 215
8.4 Conclusions ..... 221
8.5 References ..... 221

**9 Improving the level of vitamins in milk** ..... 229
*B. Graulet, INRA, France*
9.1 Introduction ..... 229
9.2 Naturally occurring vitamins in cow's milk ..... 231
9.3 Techniques to improve vitamin content of milk ..... 246
9.4 Conclusions ..... 247
9.5 References ..... 248

**10 Managing the environmental impact of the dairy industry: the business case for sustainability** ..... 252
*R. Pagan, N. Price and P. Prasad, The University of Queensland, Australia*
10.1 Environmental challenges facing the dairy industry ..... 252
10.2 The environmental impacts of dairy farming ..... 253
10.3 The environmental impacts of dairy processing ..... 263
10.4 Conclusions ..... 276
10.5 References ..... 276

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

**Part III Improving particular milks and milk-based products**

**11 Improving organic milk** ..... 283
*R. Weller, Aberystwyth, UK*
11.1 Introduction ..... 283
11.2 The key factors affecting the quality of organic milk ..... 284
11.3 Management and husbandry techniques to improve the quality of organic milk ..... 286
11.4 Future trends that may influence the quality of organic milk . 299
11.5 References ..... 300

**12 Improving goat milk** ..... 304
*Y. Park, Fort Valley State University, USA*
12.1 Introduction: key issues in improving goat milk ..... 304
12.2 Production of quality goat milk ..... 305
12.3 Factors affecting quality of goat milk ..... 316
12.4 Developments in processing techniques for goat milk ..... 323
12.5 Improving goat milk production ..... 332
12.6 Sources of further information and advice ..... 338
12.7 References and further reading ..... 339

**13 Improving the quality and safety of sheep milk** ..... 347
*R. Bencini, The University of Western Australia, Australia and A. Stanislao Atzori, A. Nudda, G. Battacone and G. Pulina, Università degli Studi di Sassari, Italy*
13.1 Introduction: a historical perspective ..... 347
13.2 Processing of sheep milk ..... 348
13.3 Factors affecting the quality of sheep milk ..... 349
13.4 Physiological factors affecting the quality of sheep milk .... 352
13.5 Management factors affecting the quality of sheep milk ..... 354
13.6 Improving sheep milk production ..... 359
13.7 Management of milking ewes ..... 374
13.8 Developments in processing techniques for sheep milk ..... 381
13.9 Sources of further information and advice ..... 382
13.10 References and further reading ..... 382

**14 Improving buffalo milk** ..... 402
*M. Guo, University of Vermont, USA and G. Hendricks, University of Massachusetts, USA*
14.1 Introduction ..... 403
14.2 Chemical composition ..... 404
14.3 Milk products ..... 406
14.4 Dairy management and milk production ..... 408
14.5 Feeding management ..... 409

© Woodhead Publishing Limited, 2010

Downloaded from Vet-eBooks.com

14.6 Factors that influence the yield and composition of buffalo milk ..... 410
14.7 Factors to consider for improving milk production and reproductive capacity of buffalo ..... 412
14.8 References ..... 414

**15 Milk quality requirements for yoghurt-making** ..... 417
*R. K. Robinson, formerly of The University of Reading, UK and M. S. Y. Haddadin, University of Jordan, Jordan*
15.1 Introduction ..... 417
15.2 Base milk for yoghurt-making ..... 419
15.3 Establishing the conditions for coagulation ..... 423
15.4 Formation and structure of yoghurt coagulum ..... 424
15.5 Factors that affect coagulation ..... 426
15.6 Final steps in the process ..... 428
15.7 Future trends ..... 429
15.8 References and further reading ..... 430

**16 Milk quality requirements for cheesemaking** ..... 433
*S. Skeie, Norwegian University of Life Sciences, Norway*
16.1 Introduction ..... 433
16.2 Range of milks used in cheesemaking ..... 434
16.3 Effects of milk on cheesemaking, yield and quality ..... 435
16.4 Influence of milk preparation for its cheesemaking properties and for cheese quality ..... 442
16.5 Future trends ..... 446
16.6 Sources of further information and advice ..... 446
16.7 References ..... 447

**17 Trends in infant formulas: a dairy perspective** ..... 454
*R. Floris, T. Lambers, A. Alting and J. Kiers, NIZO food research B.V., The Netherlands*
17.1 Introduction ..... 455
17.2 Human milk ..... 455
17.3 Humanization of infant food ..... 459
17.4 Towards optimized composition: analytical tools and models 463
17.5 Infant food and allergenicity ..... 463
17.6 Other beneficial properties of milk protein hydrolysates ..... 466
17.7 Conclusions ..... 469
17.8 References ..... 470

**18 Applications of milk components in products other than foods** 475
*J.-L. Audic and B. Chaufer, Université de Rennes, France*
18.1 Introduction ..... 475

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

18.2 Non-food uses of major components of milk: a short review ..... 476
18.3 Conclusions ..... 483
18.4 References ..... 484

*Index* ..... 490

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

# Contributor contact details

(* = main contact)

## Chapter 1

Dr H. Bishop MacDonald
Nutrisphere
118 Grey Fox Drive
Carp, Ontario
Canada K0A 1L0
E-mail: helyn.mac@nutrisphere.ca

## Chapter 2

Dr M. de Vrese*, Dr M. Pfeuffer, Dr N. Roos, Dr K. Scholz-Ahrens and Professor Dr J. Schrezenmeir
Institute of Physiology and Biochemistry of Nutrition
Max Rubner-Institut (MRI) – Federal Research Institute of Nutrition and Food
Hermann-Weigmann-Straße
124103 Kiel
Germany
E-mail: michael.devrese@mri.bund.de

## Chapter 3

Dr M. Boland
Riddet Institute
Massey University
New Zealand
E-mail: mikeb@xtra.co.nz

## Chapter 4

M. Heyndrickx*, S. Marchand, V. De Jonghe, K. Smet, K. Coudijzer and J. De Block
Institute for Agricultural and Fisheries Research (ILVO)
Technology & Food Sciences
Brusselsesteenweg 370
B-9090 Melle
Belgium
E-mail: Marc.Heyndrickx@ilvo.vlaanderen.be

© Woodhead Publishing Limited, 2010

Downloaded From Vet-x-Books.com

## Chapter 5

Professor M.G. Kontominas
Laboratory of Food Chemistry and Technology
Department of Chemistry
University of Ioannina
45110 Greece
E-mail: mkontomi@cc.uoi.gr

## Chapter 6

K.W. Chapman
Department of Food Science
Stocking Hall
Cornell University
Ithaca, NY 14853
USA
E-mail: kwc3@cornell.edu

## Chapter 7

K.R. Cadwallader
Department of Food Science and Human Nutrition
University of Illinois
1302 W. Pennsylvania Avenue
Urbana, IL 61801
USA
E-mail: cadwlldr@illinois.edu

## Chapter 8

Dr F. Gaucheron
UMR1253 Science et Technologie du Lait et de l'œuf
INRA – Agrocampus Ouest
65 rue de Saint Brieuc
35042 Rennes Cedex
France
E-mail: Frederic.gaucheron@rennes.inra.fr

## Chapter 9

Dr Benoit Graulet
UR1213 – Research Unit on Herbivores
INRA
Clermont-Ferrand/Theix Research Centre
F-63122 Saint Genès Champanelle
France
E-mail: benoit.graulet@clermont.inra.fr

## Chapter 10

R. Pagan, N. Price and P. Prasad*
School of Geography, Planning and Environmental Management
The University of Queensland
St Lucia
Australia
E-mail: p.prasad@uq.edu.au

## Chapter 11

Richard F. Weller
41 Cefn Esgair
Llanbadarn Fawr
Aberystwyth
Ceredigion SY23 3JG
UK
E-mail: weller200@btinternet.com

## Chapter 12

Professor Y.W. Park
Georgia Small Ruminant Research and Extension Center
Fort Valley State University
Fort Valley, GA 31030
USA
E-mail: parky@fvsu.edu

© Woodhead Publishing Limited, 2010

Downloaded From Vet-x-Books.com

## Chapter 13

Roberta Bencini
M092
The University of Western Australia
35 Stirling Hwy
Nedlands
Western Australia 6009
Australia
E-mail: rbencini@animals.uwa.edu.au

## Chapter 14

Professor M.R. Guo*
Department of Nutrition and Food Sciences
University of Vermont
USA
E-mail: Ming.R.Guo@uvm.edu

Dr G. Hendricks
Medical School
University of Massachusetts
USA

## Chapter 15

R.K. Robinson (deceased)
Formerly of the University of Reading
Whiteknights
PO Box 217
Reading RG6 6AH
UK

M.S.Y. Haddadin*
University of Jordan
Amman
Jordan
E-mail: hadadin_malik@hotmail.com

## Chapter 16

Professor S.B. Skeie
Norwegian University of Life Sciences
Department of Chemistry, Biotechnology and Food Science
PO Box 5003
N-1432 Ås
Norway
E-mail: siv.skeie@umb.no

## Chapter 17

R. Floris, T.T. Lambers, A. Alting and J. Kiers
NIZO food research B.V.
Kernhemseweg 2
6718 ZB, Ede
The Netherlands
E-mail: rene.floris@nizo.nl

## Chapter 18

Jean-Luc Audic* and Bernard Chaufer
Laboratoire Chimie et Ingénierie des Procédés (CIP) UMR CNRS 6226
Université de Rennes 1 – ENSCR
Avenue du Général Leclerc
CS 50837
35708 Rennes Cedex 7
France
E-mail: jean-luc.audic@univ-rennes1.fr
bernard.chaufer@univ-rennes1.fr

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

Downloaded From Vet-eBooks.com

# Woodhead Publishing Series in Food Science, Technology and Nutrition

1 **Chilled foods: a comprehensive guide** *Edited by C. Dennis and M. Stringer*
2 **Yoghurt: science and technology** *A. Y. Tamime and R. K. Robinson*
3 **Food processing technology: principles and practice** *P. J. Fellows*
4 **Bender's dictionary of nutrition and food technology Sixth edition** *D. A. Bender*
5 **Determination of veterinary residues in food** *Edited by N. T. Crosby*
6 **Food contaminants: sources and surveillance** *Edited by C. Creaser and R. Purchase*
7 **Nitrates and nitrites in food and water** *Edited by M. J. Hill*
8 **Pesticide chemistry and bioscience: the food–environment challenge** *Edited by G. T. Brooks and T. Roberts*
9 **Pesticides: developments, impacts and controls** *Edited by G. A. Best and A. D. Ruthven*
10 **Dietary fibre: chemical and biological aspects** *Edited by D. A. T. Southgate, K. W. Waldron, I. T. Johnson and G. R. Fenwick*
11 **Vitamins and minerals in health and nutrition** *M. Tolonen*
12 **Technology of biscuits, crackers and cookies Second edition** *D. Manley*
13 **Instrumentation and sensors for the food industry** *Edited by E. Kress-Rogers*
14 **Food and cancer prevention: chemical and biological aspects** *Edited by K. W. Waldron, I. T. Johnson and G. R. Fenwick*
15 **Food colloids: proteins, lipids and polysaccharides** *Edited by E. Dickinson and B. Bergenstahl*
16 **Food emulsions and foams** *Edited by E. Dickinson*
17 **Maillard reactions in chemistry, food and health** *Edited by T. P. Labuza, V. Monnier, J. Baynes and J. O'Brien*
18 **The Maillard reaction in foods and medicine** *Edited by J. O'Brien, H. E. Nursten, M. J. Crabbe and J. M. Ames*
19 **Encapsulation and controlled release** *Edited by D. R. Karsa and R. A. Stephenson*
20 **Flavours and fragrances** *Edited by A. D. Swift*
21 **Feta and related cheeses** *Edited by A. Y. Tamime and R. K. Robinson*
22 **Biochemistry of milk products** *Edited by A. T. Andrews and J. R. Varley*
23 **Physical properties of foods and food processing systems** *M. J. Lewis*

© Woodhead Publishing Limited, 2010

Downloaded From Vet-x-Books.com

24 **Food irradiation: a reference guide** *V. M. Wilkinson and G. Gould*
25 **Kent's technology of cereals: an introduction for students of food science and agriculture Fourth edition** *N. L. Kent and A. D. Evers*
26 **Biosensors for food analysis** *Edited by A. O. Scott*
27 **Separation processes in the food and biotechnology industries: principles and applications** *Edited by A. S. Grandison and M. J. Lewis*
28 **Handbook of indices of food quality and authenticity** *R. S. Singhal, P. K. Kulkarni and D. V. Rege*
29 **Principles and practices for the safe processing of foods** *D. A. Shapton and N. F. Shapton*
30 **Biscuit, cookie and cracker manufacturing manuals Volume 1: ingredients** *D. Manley*
31 **Biscuit, cookie and cracker manufacturing manuals Volume 2: biscuit doughs** *D. Manley*
32 **Biscuit, cookie and cracker manufacturing manuals Volume 3: biscuit dough piece forming** *D. Manley*
33 **Biscuit, cookie and cracker manufacturing manuals Volume 4: baking and cooling of biscuits** *D. Manley*
34 **Biscuit, cookie and cracker manufacturing manuals Volume 5: secondary processing in biscuit manufacturing** *D. Manley*
35 **Biscuit, cookie and cracker manufacturing manuals Volume 6: biscuit packaging and storage** *D. Manley*
36 **Practical dehydration Second edition** *M. Greensmith*
37 **Lawrie's meat science Sixth edition** *R. A. Lawrie*
38 **Yoghurt: science and technology Second edition** *A. Y. Tamime and R. K. Robinson*
39 **New ingredients in food processing: biochemistry and agriculture** *G. Linden and D. Lorient*
40 **Benders' dictionary of nutrition and food technology Seventh edition** *D. A. Bender and A. E. Bender*
41 **Technology of biscuits, crackers and cookies Third edition** *D. Manley*
42 **Food processing technology: principles and practice Second edition** *P. J. Fellows*
43 **Managing frozen foods** *Edited by C. J. Kennedy*
44 **Handbook of hydrocolloids** *Edited by G. O. Phillips and P. A. Williams*
45 **Food labelling** *Edited by J. R. Blanchfield*
46 **Cereal biotechnology** *Edited by P. C. Morris and J. H. Bryce*
47 **Food intolerance and the food industry** *Edited by T. Dean*
48 **The stability and shelf life of food** *Edited by D. Kilcast and P. Subramaniam*
49 **Functional foods: concept to product** *Edited by G. R. Gibson and C. M. Williams*
50 **Chilled foods: a comprehensive guide Second edition** *Edited by M. Stringer and C. Dennis*
51 **HACCP in the meat industry** *Edited by M. Brown*
52 **Biscuit, cracker and cookie recipes for the food industry** *D. Manley*
53 **Cereals processing technology** *Edited by G. Owens*
54 **Baking problems solved** *S. P. Cauvain and L. S. Young*
55 **Thermal technologies in food processing** *Edited by P. Richardson*
56 **Frying: improving quality** *Edited by J. B. Rossell*
57 **Food chemical safety Volume 1: contaminants** *Edited by D. Watson*
58 **Making the most of HACCP: learning from others' experience** *Edited by T. Mayes and S. Mortimore*
59 **Food process modelling** *Edited by L. M. M. Tijskens, M. L. A. T. M. Hertog and B. M. Nicolaï*

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

60 **EU food law: a practical guide** *Edited by K. Goodburn*
61 **Extrusion cooking: technologies and applications** *Edited by R. Guy*
62 **Auditing in the food industry: from safety and quality to environmental and other audits** *Edited by M. Dillon and C. Griffith*
63 **Handbook of herbs and spices Volume 1** *Edited by K. V. Peter*
64 **Food product development: maximising success** *M. Earle, R. Earle and A. Anderson*
65 **Instrumentation and sensors for the food industry Second edition** *Edited by E. Kress-Rogers and C. J. B. Brimelow*
66 **Food chemical safety Volume 2: additives** *Edited by D. Watson*
67 **Fruit and vegetable biotechnology** *Edited by V. Valpuesta*
68 **Foodborne pathogens: hazards, risk analysis and control** *Edited by C. de W. Blackburn and P. J. McClure*
69 **Meat refrigeration** *S. J. James and C. James*
70 **Lockhart and Wiseman's crop husbandry Eighth edition** *H. J. S. Finch, A. M. Samuel and G. P. F. Lane*
71 **Safety and quality issues in fish processing** *Edited by H. A. Bremner*
72 **Minimal processing technologies in the food industries** *Edited by T. Ohlsson and N. Bengtsson*
73 **Fruit and vegetable processing: improving quality** *Edited by W. Jongen*
74 **The nutrition handbook for food processors** *Edited by C. J. K. Henry and C. Chapman*
75 **Colour in food: improving quality** *Edited by D MacDougall*
76 **Meat processing: improving quality** *Edited by J. P. Kerry, J. F. Kerry and D. A. Ledward*
77 **Microbiological risk assessment in food processing** *Edited by M. Brown and M. Stringer*
78 **Performance functional foods** *Edited by D. Watson*
79 **Functional dairy products Volume 1** *Edited by T. Mattila-Sandholm and M. Saarela*
80 **Taints and off-flavours in foods** *Edited by B. Baigrie*
81 **Yeasts in food** *Edited by T. Boekhout and V. Robert*
82 **Phytochemical functional foods** *Edited by I. T. Johnson and G. Williamson*
83 **Novel food packaging techniques** *Edited by R. Ahvenainen*
84 **Detecting pathogens in food** *Edited by T. A. McMeekin*
85 **Natural antimicrobials for the minimal processing of foods** *Edited by S. Roller*
86 **Texture in food Volume 1: semi-solid foods** *Edited by B. M. McKenna*
87 **Dairy processing: improving quality** *Edited by G Smit*
88 **Hygiene in food processing: principles and practice** *Edited by H. L. M. Lelieveld, M. A. Mostert, B. White and J. Holah*
89 **Rapid and on-line instrumentation for food quality assurance** *Edited by I. Tothill*
90 **Sausage manufacture: principles and practice** *E. Essien*
91 **Environmentally-friendly food processing** *Edited by B. Mattsson and U. Sonesson*
92 **Bread making: improving quality** *Edited by S. P. Cauvain*
93 **Food preservation techniques** *Edited by P. Zeuthen and L. Bøgh-Sørensen*
94 **Food authenticity and traceability** *Edited by M. Lees*
95 **Analytical methods for food additives** *R. Wood, L. Foster, A. Damant and P. Key*
96 **Handbook of herbs and spices Volume 2** *Edited by K. V. Peter*
97 **Texture in food Volume 2: solid foods** *Edited by D. Kilcast*
98 **Proteins in food processing** *Edited by R. Yada*
99 **Detecting foreign bodies in food** *Edited by M. Edwards*

© Woodhead Publishing Limited, 2010

Downloaded From Vet-eBooks.com

100 **Understanding and measuring the shelf-life of food** *Edited by R. Steele*
101 **Poultry meat processing and quality** *Edited by G. Mead*
102 **Functional foods, ageing and degenerative disease** *Edited by C. Remacle and B. Reusens*
103 **Mycotoxins in food: detection and control** *Edited by N. Magan and M. Olsen*
104 **Improving the thermal processing of foods** *Edited by P. Richardson*
105 **Pesticide, veterinary and other residues in food** *Edited by D. Watson*
106 **Starch in food: structure, functions and applications** *Edited by A.-C. Eliasson*
107 **Functional foods, cardiovascular disease and diabetes** *Edited by A. Arnoldi*
108 **Brewing: science and practice** *D. E. Briggs, P. A. Brookes, R. Stevens and C. A. Boulton*
109 **Using cereal science and technology for the benefit of consumers: proceedings of the 12th International ICC Cereal and Bread Congress, 24–26 May, 2004, Harrogate, UK** *Edited by S. P. Cauvain, L. S. Young and S. Salmon*
110 **Improving the safety of fresh meat** *Edited by J. Sofos*
111 **Understanding pathogen behaviour in food: virulence, stress response and resistance** *Edited by M. Griffiths*
112 **The microwave processing of foods** *Edited by H. Schubert and M. Regier*
113 **Food safety control in the poultry industry** *Edited by G. Mead*
114 **Improving the safety of fresh fruit and vegetables** *Edited by W. Jongen*
115 **Food, diet and obesity** *Edited by D. Mela*
116 **Handbook of hygiene control in the food industry** *Edited by H. L. M. Lelieveld, M. A. Mostert and J. Holah*
117 **Detecting allergens in food** *Edited by S. Koppelman and S. Hefle*
118 **Improving the fat content of foods** *Edited by C. Williams and J. Buttriss*
119 **Improving traceability in food processing and distribution** *Edited by I. Smith and A. Furness*
120 **Flavour in food** *Edited by A. Voilley and P. Etievant*
121 **The Chorleywood bread process** *S. P. Cauvain and L. S. Young*
122 **Food spoilage microorganisms** *Edited by C. de W. Blackburn*
123 **Emerging foodborne pathogens** *Edited by Y. Motarjemi and M. Adams*
124 **Benders' dictionary of nutrition and food technology Eighth edition** *D. A. Bender*
125 **Optimising sweet taste in foods** *Edited by W. J. Spillane*
126 **Brewing: new technologies** *Edited by C. Bamforth*
127 **Handbook of herbs and spices Volume 3** *Edited by K. V. Peter*
128 **Lawrie's meat science Seventh edition** *R. A. Lawrie in collaboration with D. A. Ledward*
129 **Modifying lipids for use in food** *Edited by F. Gunstone*
130 **Meat products handbook: practical science and technology** *G. Feiner*
131 **Food consumption and disease risk: consumer–pathogen interactions** *Edited by M. Potter*
132 **Acrylamide and other hazardous compounds in heat-treated foods** *Edited by K. Skog and J. Alexander*
133 **Managing allergens in food** *Edited by C. Mills, H. Wichers and K. Hoffman-Sommergruber*
134 **Microbiological analysis of red meat, poultry and eggs** *Edited by G. Mead*
135 **Maximising the value of marine by-products** *Edited by F. Shahidi*
136 **Chemical migration and food contact materials** *Edited by K. Barnes, R. Sinclair and D. Watson*
137 **Understanding consumers of food products** *Edited by L. Frewer and H. van Trijp*
138 **Reducing salt in foods: practical strategies** *Edited by D. Kilcast and F. Angus*
139 **Modelling microrganisms in food** *Edited by S. Brul, S. Van Gerwen and M. Zwietering*

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

140 **Tamime and Robinson's Yoghurt: science and technology Third edition** *A. Y. Tamime and R. K. Robinson*
141 **Handbook of waste management and co-product recovery in food processing: Volume 1** *Edited by K. W. Waldron*
142 **Improving the flavour of cheese** *Edited by B. Weimer*
143 **Novel food ingredients for weight control** *Edited by C. J. K. Henry*
144 **Consumer-led food product development** *Edited by H. MacFie*
145 **Functional dairy products Volume 2** *Edited by M. Saarela*
146 **Modifying flavour in food** *Edited by A. J. Taylor and J. Hort*
147 **Cheese problems solved** *Edited by P. L. H. McSweeney*
148 **Handbook of organic food safety and quality** *Edited by J. Cooper, C. Leifert and U. Niggli*
149 **Understanding and controlling the microstructure of complex foods** *Edited by D. J. McClements*
150 **Novel enzyme technology for food applications** *Edited by R. Rastall*
151 **Food preservation by pulsed electric fields: from research to application** *Edited by H. L. M. Lelieveld and S. W. H. de Haan*
152 **Technology of functional cereal products** *Edited by B. R. Hamaker*
153 **Case studies in food product development** *Edited by M. Earle and R. Earle*
154 **Delivery and controlled release of bioactives in foods and nutraceuticals** *Edited by N. Garti*
155 **Fruit and vegetable flavour: recent advances and future prospects** *Edited by B. Brückner and S. G. Wyllie*
156 **Food fortification and supplementation: technological, safety and regulatory aspects** *Edited by P. Berry Ottaway*
157 **Improving the health-promoting properties of fruit and vegetable products** *Edited by F. A. Tomás-Barberán and M. I. Gil*
158 **Improving seafood products for the consumer** *Edited by T. Børresen*
159 **In-pack processed foods: improving quality** *Edited by P. Richardson*
160 **Handbook of water and energy management in food processing** *Edited by J. Klemeš, R. Smith and J-K Kim*
161 **Environmentally compatible food packaging** *Edited by E. Chiellini*
162 **Improving farmed fish quality and safety** *Edited by Ø. Lie*
163 **Carbohydrate-active enzymes** *Edited by K-H Park*
164 **Chilled foods: a comprehensive guide Third edition** *Edited by M. Brown*
165 **Food for the ageing population** *Edited by M. M. Raats, C. P. G. M. de Groot and W. A. Van Staveren*
166 **Improving the sensory and nutritional quality of fresh meat** *Edited by J. P. Kerry and D. A. Ledward*
167 **Shellfish safety and quality** *Edited by S. E. Shumway and G. E. Rodrick*
168 **Functional and speciality beverage technology** *Edited by P. Paquin*
169 **Functional foods: principles and technology** *M. Guo*
170 **Endocrine-disrupting chemicals in food** *Edited by I. Shaw*
171 **Meals in science and practice: interdisciplinary research and business applications** *Edited by H. L. Meiselman*
172 **Food constituents and oral health: current status and future prospects** *Edited by M. Wilson*
173 **Handbook of hydrocolloids Second edition** *Edited by G. O. Phillips and P. A. Williams*
174 **Food processing technology: principles and practice Third edition** *P. J. Fellows*
175 **Science and technology of enrobed and filled chocolate, confectionery and bakery products** *Edited by G. Talbot*

© Woodhead Publishing Limited, 2010

Downloaded From Vet-x-Books.com

176 **Foodborne pathogens: hazards, risk analysis and control Second edition** *Edited by C. de W. Blackburn and P. J. McClure*
177 **Designing functional foods: measuring and controlling food structure breakdown and absorption** *Edited by D. J. McClements and E. A. Decker*
178 **New technologies in aquaculture: improving production efficiency, quality and environmental management** *Edited by G. Burnell and G. Allan*
179 **More baking problems solved** *S. P. Cauvain and L. S. Young*
180 **Soft drink and fruit juice problems solved** *P. Ashurst and R. Hargitt*
181 **Biofilms in the food and beverage industries** *Edited by P. M. Fratamico, B. A. Annous and N. W. Gunther*
182 **Dairy-derived ingredients: food and neutraceutical uses** *Edited by M. Corredig*
183 **Handbook of waste management and co-product recovery in food processing Volume 2** *Edited by K. W. Waldron*
184 **Innovations in food labelling** *Edited by J. Albert*
185 **Delivering performance in food supply chains** *Edited by C. Mena and G. Stevens*
186 **Chemical deterioration and physical instability of food and beverages** *Edited by L. Skibsted, J. Risbo and M. Andersen*
187 **Managing wine quality Volume 1: viticulture and wine quality** *Edited by A. Reynolds*
188 **Improving the safety and quality of milk Volume 1: milk production and processing** *Edited by M. Griffiths*
189 **Improving the safety and quality of milk Volume 2: improving quality in milk products** *Edited by M. Griffiths*
190 **Cereal grains: assessing and managing quality** *Edited by C. Wrigley and I. Batey*
191 **Sensory analysis for food and beverage control: a practical guide** *Edited by D. Kilcast*
192 **Managing wine quality Volume 2: oenology and wine quality** *Edited by A. Reynolds*
193 **Winemaking problems solved** *Edited by C. Butzke*
194 **Environmental assessment and management in the food industry** *Edited by U. Sonesson, J. Berlin and F. Ziegler*
195 **Consumer-driven innovation in food and personal care products** *Edited by S. Jaeger and H. MacFie*
196 **Tracing pathogens in the food chain** *Edited by S. Brul, P.M. Fratamico and T.A. McMeekin*
197 **Case studies in novel food processing technologies** *Edited by C. Doona, K Kustin and F. Feeherry*
198 **Freeze-drying of pharmaceutical and food products** *Tse-Chao Hua, Bao-Lin Liu and Hua Zhang*

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

# Preface

Since I first started my career in dairy research at the now defunct Hannah Research Institute in 1974, the dairy industry worldwide has faced many changes. With regard to food safety, it has witnessed the emergence of food-borne pathogens not previously associated with dairy products, such as *Listeria monocytogenes* and *Escherichia coli* O157:H7 along with the introduction of preventive food safety management systems (HACCP) to limit the impact of these pathogens. This year has seen the publication of the sequence of the entire cow genome, a feat that opens up innumerable possibilities. The use of this information will allow us, for example, to improve production costs through identification of traits related to feed conversion, to produce milk with specific characteristics and to impact animal welfare by selection of animals with increased disease resistance. These are just a few of the benefits the industry may reap. With more research it is also becoming apparent that milk and milk products are not the nutritional minefield that many would have us believe, in fact dairy products possess bioactive components that show substantial promise for health promotion.

It is the intent of this book to provide up-to-date coverage of several facets related to the production and processing of safe, wholesome and nutritious dairy products, not only from bovine milk but also from other domesticated ruminants. The first volume includes chapters related to milk safety and quality and focuses on the microbiological and chemical safety of raw milk and technologies for analyzing and processing milk. In the second volume, nutritional, sensory and sustainability issues are addressed as well as those associated with other milk-producing mammals and specific milk products.

I would like to thank all the contributing authors for their hard work and patience in waiting for edits. I would particularly like to acknowledge the

© Woodhead Publishing Limited, 2010

Downloaded from Vet-eBooks.com

contribution of Dr Richard Robinson, who sadly died during the production of this book. Dr Robinson was well known by all in the dairy industry for his research and the many books he edited.

On a personal note I would like to thank my wife, Susan, for her understanding and support, my two daughters, Megan and Bethan, and their respective husbands, Darren and Eric, and my four grandchildren, Rhys, Emma, Sophie and Evan, for keeping me young at heart.

I would also like to thank Dairy Farmers of Ontario for all the support they have given me over the last 20 years.

To all the readers of this book, I hope you learn from it and that it makes you realize that the proper production and processing of milk is complex and is carried out by professional and dedicated farmers and processors.

© Woodhead Publishing Limited, 2010

Downloaded From Vet-eBooks.com

# 1

# The role of milk in the diet

**H. Bishop MacDonald, Nutrisphere, Canada**

**Abstract:** The Bible speaks of milk and honey and, interestingly enough, these two are the only foods whose sole purpose is as food. Milk, unfortunately, has become the victim of much mythology that has served to obscure its extremely important role in supplying nutrition to most of the world's inhabitants. This chapter will look at the variety of mammals from which milk is derived worldwide, the variety of forms in which it is consumed and, most important, the nutritional contribution that milk makes to the overall well-being of humans. Macronutrients and micronutrients will be discussed along with milk's impact in various stages of life as well as the likely nutritional status of those who abstain from consuming milk in any form.

**Key words:** milk, dairy, nutrition, nutrient deficiencies.

IP Address: 129.132.208.100

## 1.1 Introduction

Although it has not been possible to pinpoint the exact date at which milk from various species was used to nourish humans, it is a pretty safe bet that as soon as animals were domesticated (about 9000 BC), their milk, in a variety of forms, was used as food for humans (McGee, 1984). Not only were the animals of longer use for food when kept alive instead of being slaughtered for their meat, ruminants (the primary source) thrived on dry grass thereby converting an otherwise useless commodity into a nutritious product. Today we are most accustomed to thinking of cows as the major supplier of milk (with goats becoming increasingly popular as sources of milk and cheese), but they were neither the first nor the only source. Along with milk from goats and sheep, humans have used at various times in history the milk from camels, yaks, water buffalo, reindeer, donkeys, horses and

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

zebra, to name but a few of the mammals that have been called into service. Fluid milk is consumed both as a food and as a thirst-quenching beverage both in its fresh form and as a fermented and/or cultured product. Its transformation into cheese and yoghurt is arguably the first instance of a 'convenience' food, certainly for the camel-drivers that took advantage of this spirit of cooperation between food and bacteria. For millennia now mammalian milks and milk products have been a staple in the diet of most cultures. To say that milk is nature's most nearly perfect food is not stretching the truth, as can readily be seen by examining the myriad of nutrients that constitute this amazing foodstuff.

This chapter will examine milk consumption worldwide, including source animals, the variety of forms in which milk is consumed, and the various ethnic traditions that dictate its use and the cultural impact of milk as a food. The important role of milk in the diet will be addressed with particular attention to the nutritional impact of milk on the well-being of humans. The part played by milk at various stages in the life cycle will be discussed as will the likely nutritional status of those who, either by intent or circumstance, abstain from consuming milk in any form. Finally, there will be a list of reliable sources of information that readers can access to further their knowledge of milk and milk products and a summation of the author's advice regarding recommended consumption of these foods.

## 1.2 Milk consumption worldwide

IP Address: 129.132.208.100

Tempting though it is to generalize about worldwide milk consumption (highest in Scandinavia, lowest in China, that kind of thing), in fact milk consumption in any country is dependent on many factors. Some of those factors are things like age, sex, ethnicity, and a combination of all three. The United States and Canada, for example, are considered dairying countries with a relatively high level of milk consumption. But who is drinking the milk, and what kind of milk are they drinking? Many factors contribute to milk consumption (Brewer *et al.*, 1999) including beliefs, attitudes and sensory evaluation. These same authors found that milk drinking among women was actually quite low, with 23 of the 100 subjects admitting that their milk consumption was close to non-existent. Researchers in New Zealand (Gulliver and Horwath, 2001) found a similar situation in their country and attributed the situation to factors including the belief that milk is fattening and perceived lactose intolerance. Male adolescents, on the other hand, have been shown (Larson *et al.*, 2003) to have a higher intake of dairy products and calcium than their female counterparts.

### 1.2.1 Variety of forms and sources of milk and milk products in disparate cultures

Fond though the young western male might be of a cool glass of milk, he might decline the offer if the source of that milk is other than a cow. While goat's milk

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

and sheep's milk are becoming more popular in northern Europe and North America, in many other parts of the world those milks have long been traditional beverages. What further distinguishes one culture from another is not just the source of their milk, but the form in which it is consumed. Probably first used in the Middle East, Turkey and Iran, one can easily imagine yoghurt developing spontaneously as milk was transported in goat skin bags as nomads crossed the desert. The combination of naturally occurring bacteria, sun and constant churning from the camels' marching were a sure-fire bet to produce the first fermented milk product. On the other hand, if the saddlebags were made from a young goat's stomach, then the rennin it contained would see the resulting product become what we know today as cheese. Other forms that serve as the delivery system for milk's goodness include fermented milks like kefir, buttermilk, cottage cheese and whey.

In a nutshell, then, various cultures have traditionally used various forms of milk, and that use depended a good deal on the age and stage of life, ranging from the feeding of fluid milk to children, to widespread use of cheese and yoghurt, to the burgeoning idea that yoghurt itself, by virtue of various bacteria, confers health-promoting advantages.

## 1.3 Nutritional benefits of milk

If we were in the middle of the twentieth century a discussion of the benefits of milk would hardly be deemed necessary: practically everyone accepted that milk had a place in a healthy diet – although excessive intake of any particular food was discouraged. But then food consumption began to lean toward 'political correctness' and milk avoidance became, in some circles, to be seen not only as healthy (all that cholesterol and saturated fats!) but also as a solution to world hunger (because grazing cows were consuming food that would otherwise go to humans) and a safeguard for Mother Earth (WHO Technical Report Series 916, World Health Organization, 2003). Various groups aggressively attacked the use of animals and their by-products, and the WHO/FAO Report *Diet, Nutrition and the Prevention of Chronic Diseases*, in a memorable expression of a preference for illusion over evidence, laid the blame for the increasing incidence of non-communicable diseases in developing countries at the hooves of cows and their milk (WHO Technical Report Series 916). Before addressing, then, the myriad ways in which milk can contribute to the overall well-being of humans, let's first look at the natural resources expended on the raising of mammals for their milk. Essentially, world hunger is a political and economic problem, not one of production. Beyond that, most agricultural land in the world, nearly 70%, produces grass. Grass isn't a food for humans, but for ruminants that can convert grass and other forages into human food. Forages also play a soil conservation role on cultivated land. Furthermore, the grains that ruminants eat are different: livestock eat a lower grade that isn't suitable for human consumption (Pimentel, 1980; Pimentel and Pimentel, 2003). This very brief discussion certainly doesn't

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

lay the issue to rest; I'll leave that to others with more expertise on the subject elsewhere in this book.

### 1.3.1 Milk's macronutrients: protein, fat and carbohydrate

*Milk protein*

Fluid milk contains 30–35 g/litre of total protein. This protein is of high nutritive quality with a biological protein value of 0.9 compared to the 1.0 of whole egg protein (a slight inferiority in sulphur amino acids denies milk protein first place). The major proteins of milk include caseins, lactoglobulins and lactalbumins, and are classified as either caseins (82%) or whey proteins (18%). Due to their structure caseins are readily digested and absorbed by the human gastrointestinal tract. The accumulation of proteins during growth and development and the maintenance of tissue proteins in the adult are important for ensuring an individual's nutritional well-being (Munro and Crim, 1988).

Obviously, the best source of protein for a newborn infant is breast milk. As the child grows, however, and other foods supply the necessary protein, cows' (or other mammals') milk takes on added importance, especially owing to its content of essential amino acids. This 'high quality protein' or 'complete protein' refers to the ratio of amino acids appropriate to the body's needs. Milk contains all eight of the essential amino acids required from food that our body cannot manufacture itself. The milk amino acid pattern of distribution resembles the pattern required by humans, with the relative surplus of the amino acid lysine making milk protein very valuable in vegetarian diets where it can complement low-lysine vegetable proteins. Not only are milk's proteins valuable in and of themselves, they play an important role in enhancing the otherwise poorer quality proteins found in cereal and vegetable products. They do this by supplying the amino acids in which plant proteins are usually deficient.

The 'western' diet is frequently accused of having 'too much' protein and milk is identified as one of the culprits. The consequences of 'too much' are seldom spelled out, but in the case of dairy products the downside is usually determined to be osteoporosis (Feskanich *et al.*, 1996). While a high protein diet may not actually be necessary, it would seem to be natural. Evidence indicates (Harris, 1986; Morris, 1994) that evolving hominids and primitive hunter-gatherers had higher protein intakes than do humans living in industrialized nations today. The evolving hominid, however, also had a very high calcium intake, which allowed it to compensate very easily for the additional excretory calcium loss associated with a high protein intake (Heaney, 1998). In fact, the potential impact of protein on osteoporosis exists only if calcium intake is inadequate. Research (Bowen *et al.*, 2004) indicated a high dairy protein, high calcium diet can minimize bone turnover in overweight adults who follow a weight-loss programme. A very recent study (Thorpe *et al.*, 2008) has shown that a diet rich in protein, dairy and calcium, as opposed to a conventional high-carbohydrate, energy-reduced diet, actually promoted bone health over a 12-month period. Moreover, there is far from an excessive amount of protein in

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

milk, one cup (250 mL) providing roughly 8 grams of protein. Additionally, the protein in milk has been shown (FitzGerald *et al.*, 2004) to be helpful in lowering blood pressure, while whey proteins can impact platelet aggregation (Rutherford and Gill, 2000) and might even ameliorate serum lipid profiles (Walzem *et al.*, 2002). The impact of milk consumption on the potential for blood clotting may have much to do with its protein content. One study (Caen *et al.*, 1992) looked at the positive anti-thrombotic effects of milk protein-derived peptides, and another (Bal dit Sollier *et al.*, 1996) gave evidence that three peptides from $\kappa$-casein had anti-thrombotic activity both *in vivo* and *in vitro*.

*Milk fat*

Milk fat is unique among the various fats and oils that humans consume. Most of that uniqueness has to do with its content of short-chain fatty acids that influence cellular growth while encouraging differentiation and discouraging cancerous changes (Parodi, 1997). Milk fat contains many components (to be discussed later) that potentially have beneficial effects on health. All this notwithstanding, however, the current mindset, despite much evidence to the contrary, is that dairy fat is linked to heart disease. While it is true that certain saturated fatty acids in milk fat can raise LDL levels (Mensink *et al.*, 2003), it is most likely that this elevation is offset by an equal ability to raise HDL levels (Hu and Willett, 2000). The amount of evidence that dairy fat, as part of a balanced diet, is not causative in coronary heart disease (CHD) is so great as to fill many books, which it has. This brief review will highlight some of the more pertinent studies that have focused on the issue.

In one of the many published pieces of research to come from the Nurses' Health Study (Willett *et al.*, 1993) women who ate four or more teaspoons of margarine per day were at higher risk of CHD than women who ate margarine less than once per month. That in itself is very interesting, but one of the key statements included in the published work is that the intake of butter, which is not an important source of *trans*-isomers, was not significantly associated with risk of CHD. The next year a case-control study (Ascherio *et al.*, 1994) looked at *trans*-fatty acid intake and first myocardial infarction and found that the risk was almost entirely accounted for by *trans*-fatty acid intake from partially hydrogenated vegetable fats like margarine and shortening. No significant association was seen between intakes of *trans*-isomers from ruminant fat. In 1996 data from the Health Professionals Follow-up Study showed no association between intake of saturated fat and risk of coronary heart disease after adjustment for fibre intake (Ascherio *et al.*, 1996). Those results are consistent with the possibility that the proportional increase in concentration of HDL produced by saturated fat compensates for the alleged adverse effect on total serum cholesterol concentration. That same year saw the publication of the Honolulu Heart Program study (Abbott *et al.*, 1996) in which those who consumed less than two glasses of milk per day had twice the rate of strokes as those who consumed two or more glasses of milk. Studying a cohort of 21,930 men (Pietinen *et al.*, 1997), researchers found an association between CHD death and *trans*-fatty acid intakes, but no

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

association between such deaths and animal-origin saturated fatty acids. In a 20-year follow-up of over 800 men from the Framingham Study (Gillman *et al.*, 1997a), the incidence of strokes was found to be lower in those men whose intakes of total fat, saturated and monounsaturated fatty acids were the highest. Intakes of fat and types of fat were not related to the incidence of the combined outcome of all cardiovascular disease. The same investigators (Gillman *et al.*, 1997b) found that in a Framingham cohort of 832 men, the group with the highest incidence of CHD consumed the least amount of butter, while that with the lowest incidence consumed the most butter.

In the Prospective Assessment of Coronary Heart Disease Risk Factors: the NHANES I Epidemiologic Follow-up Study; 16-Year Follow-up (Gartside *et al.*, 1998), cheese intake was found to be significantly inversely related to CHD events. The authors found this result to be puzzling since they, and others, would normally expect the relatively high levels of saturated fats and cholesterol in cheese to lead to an increase in CHD events. A review of ecological, case-control and cohort studies on dietary fat and coronary vascular disease (Ravnskov, 1998) saw no harmful effect of saturated fat and questioned the hypothesis that a diet rich in those fats leads to atherosclerosis. In a somewhat similar vein researchers (Fehily *et al.*, 1993) found no association between animal fat consumption and ischaemic heart disease. Smedman *et al.* (1999) looked at 70-year-old Finnish men and found an inverse association between the intake of milk products (including full-fat dairy) and BMI, waist circumference, and LDL:HDL ratio. A positive association between intake of milk products and HDL cholesterol and apolipoprotein A-1 levels was observed. A case-control study of 100 post-heart attack patients in Norway (Pedersen *et al.*, 2000) examined the fat from adipose tissue and found that those with high *trans*-fat content had a significantly higher risk of myocardial infarction; saturated fats were not associated. In a 25-year study of more than 5700 men between the ages of 35 and 64 (Ness *et al.*, 2001) it was found that death from heart disease was 8% lower among men who drank more than a third of a pint (170 mL) of milk per day compared to those who drank less. This was at a time when most milk consumed was full-fat. The study also showed that death from all causes, including cancer and stroke, was 10% lower among milk drinkers compared with non-drinkers.

Samuelson *et al.* (2001) examined the relationship between the dietary content of saturated fatty acids with a chain length of four to 15 carbon atoms (mainly from milk fat) and serum concentrations of cholesterol and ApoB and found a significant inverse association. A short report (Tavani *et al.*, 2002) looked at 507 cases and 478 controls to determine the impact of milk and dairy product consumption on the risk of acute myocardial infarction. They found that drinkers of any kind of milk not only did not experience increased risk, but in fact with an intake equal to or greater than seven cups per week, had an age and sex adjusted odds ratio of 0.78 compared to non-drinkers. Writing in the *American Journal of Clinical Nutrition*, Mozaffarian *et al.* (2004) found less progression of coronary atherosclerosis in postmenopausal women whose intake

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

of saturated fats was higher, and a greater progression in those whose carbohydrate consumption was higher. Swedish researchers (Warensjö *et al.*, 2004) estimated milk-fat intake based on the proportions of pentadecanoic and heptadecanoic acids in serum lipid esters. They found a negative association with cardiovascular risk factors and no increase of risk for a first acute myocardial infarction. Another group (Biong *et al.*, 2008) has recently replicated these findings. Again in 2004 another group of Swedes (Sjogren *et al.*, 2004) looking at particle size distribution and risk for coronary heart disease found that the fatty acids derived from dairy foods were associated with fewer small, dense LDL particles.

A discussion of LDL particle size distribution helps highlight the problems inherent in oversimplifying the complex relationship between diet (particularly saturated fat) and heart health. Research published in the *Journal of the American Medical Association* (Austin *et al.*, 1988) looked at LDL subclass patterns in a case-control study and found a threefold increased risk of myocardial infarction in patients whose LDL subclass pattern included a preponderance of small, dense LDL particles. As noted above, fatty acids from dairy foods have been associated with fewer small, dense LDL particles. A study (Dreon *et al.*, 1994) investigating a possible association between LDL subclass patterns and the response of plasma lipoprotein levels to alternating intakes of dietary carbohydrate and fat found profound differences in group responses. Those subjects with a preponderance of pattern B (small, dense LDL particles) had a significant improvement over pattern-A subjects (larger LDL particles) in response to a high-fat diet. This set the stage for further research into the impact of subclass patterns on variation in response to high-fat versus low-fat diets. To complicate matters further, Krauss (2001) showed that low-fat, high-carbohydrate diets resulted in the conversion of pattern-A men to pattern-B, in other words, a shift from large to small LDL particles. In further examining this effect of low-fat, high-carbohydrate diets on phenotype-A subjects (Krauss, 2005) it was shown that these diet-induced subclass changes are most likely gene related and that further work is needed to determine who will most benefit from which dietary modifications. Kaess *et al.* (2008) analysed heritability and linkage for HDL and LDL subclass features and hope in future to be able to identify which genes control the lipoprotein subclass distribution. One size most definitely doesn't fit all, nor is it wise to suggest that all will benefit from a diet low in milk fat. And, milk fat aside, there is evidence (Pfeuffer and Schrezenmeir, 2000) that particular bioactive substances in milk contribute to a decreased risk of cardiovascular disease.

The complications regarding milk fat don't stop there. In their haste to encourage the public to either swear off milk altogether or at least to consume only fat-free milk products (Duyff, 2006), health authorities have forgotten or ignored the accumulating evidence that milk fat actually contains substances that are health promoting! Not only has it been shown that the major, naturally occurring, *trans*-fatty acids in dairy fat (rumenic acid and vaccenic acid) possess anti-carcinogenic capability (Parodi, 1997; Belury, 2002), but such acids might

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

be anti-atherogenic as well (Parodi, 2004), possibly due to their ability to modulate inflammatory markers of atherosclerosis. Conjugated linoleic acid (CLA) is the collective term given to these positional and geometric isomers of linoleic acid, and it is found in the fat of all ruminant animals. Given the urging of the public to avoid or drastically cut down on the consumption of animal fats, it is not surprising that intake of CLA has dwindled. On top of that, the appropriate rejection of industrially produced *trans*-fatty acids has resulted in the replacement of these fats with linoleic-rich vegetable oils. The resulting situation has a public that has severely reduced its intake of a potent anti-carcinogen while at the same time dramatically increasing the intake of linoleic acid, shown to be carcinogenic when consumed in large amounts (Lands, 2008). Another component of milk, if not milk fat specifically, with anti-carcinogenic potential are the sphingolipids, more precisely sphingomyelin. This most common of the sphingolipids represents roughly one-third of the phospholipids in milk, depending on the time of year and the timing of lactation. Animal studies (Dillehay *et al.*, 1994) have suggested a benefit from dietary sphingomyelins, possibly by virtue of cell growth inhibition and apoptosis. A further entry in the milk-fat anti-carcinogen sweepstakes is a short-chain fatty acid known as butyric acid. Not only is it a strong inhibitor of proliferation and a promoter of differentiation and apoptosis (Hague and Paraskeva, 1995), but butyric acid has also been related to inactivation of cancer gene expression (Smith and German, 1995) and possibly metastasis and invasiveness of malignant tumours (Parodi, 2004).

Aside from the potential for specific constituents of milk and dairy products to reduce the risk of various cancers, there is growing evidence regarding the role of dairy itself in lowering the incidence of particular cancers. Breast cancer incidence, for example, has been shown (Knekt *et al.*,1996; Hjartåker *et al.*, 2001; Shin *et al.*, 2002) to be reduced among dairy consumers over non-consumers. The evidence pertaining to the impact of milk and its components in reducing colon cancer risk is also abundant. Newmark and Lipkin (1992), Holt (1999) and Parodi (2001) are among many investigators to link milk consumption, and/or by extension its various nutrients like calcium, vitamin D and CLA, to a significant reduction in colon cancer risk.

IP Address: 129.132.208.100

*Milk carbohydrate*

Far and away the predominant carbohydrate in milk is the sugar lactose. Contributing about a third of the energy derived from whole milk, lactose is also credited with enhancing the intestinal absorption of calcium by infants (Zeigler and Fomon, 1983). Lactose is also distinguished by having, along with milk fat, the greatest distortion regarding its impact on those who ingest it. To begin with, the anti-milk lobby has a penchant for claiming that roughly 80% (some go as high as 98%!) of the world's adults cannot digest lactose (Diamond and Diamond, 1987) . While technically the 80% figure might be factual in a global context, if you're living in Iceland (or any of the Scandinavian countries) with their predominantly Caucasian populace it has little bearing on whether or not

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

you can drink milk without fear of discomfort, since lactase insufficiency is relatively rare among those of northern European descent (Scrimshaw and Murray, 1988). If, in fact, you have limited or no lactase (the enzyme necessary for lactose digestion) production, or lactase non-persistence, then you might experience some or all of the various symptoms (gas, bloating, diarrhoea) known to accompany lactose intolerance. Might experience … but not necessarily. Research (Suarez *et al.*, 1997; Savaiano *et al.*, 2006) has shown that even those with true (as opposed to perceived) lactose maldigestion can consume up to two eight-ounce (250 mL) servings per day if they take it with other food. In addition, there seems to be widespread misunderstanding about which dairy foods are rich in lactose and which contain negligible or nil amounts. Butter, for example, is not a contributor of lactose, nor are most hard cheeses whose lactose escapes with the whey. The friendly bacteria in live-culture yoghurts do an excellent job of breaking down the lactose and, of course, lactose-free milks are available as are enzyme tablets that will provide the missing lactase. In short, there is little reason for 'lactose intolerance' to be used as an excuse for not consuming dairy products. All of that notwithstanding, those who are 'exquisitely sensitive' are best advised to be cautious about consuming milk and to consider a calcium supplement to cover their losses. Calcium deficiency, however, is just one of the problems facing those who reject milk and milk products altogether.

### 1.3.2 Minerals in milk

*Calcium*

So much has been written about the value and role of dietary calcium that one could be forgiven for thinking that the public is really clear on the issue. Not so. For one thing, people remain unclear about the bioavailability of calcium, and just what is the optimal amount of calcium to keep one healthy through all the life stages and into the golden years. The initial recommendations for calcium intake were based on a typical mixed diet, a balanced intake of each of the food groups. The experts understood that not all of the calcium in all foods reaches the blood stream with the same level of efficiency. Bioavailability is the process whereby what you see (in terms of nutrients) isn't necessarily what you get. Certain anti-nutrients can reduce the amount of a specific nutrient that the body can absorb. In the case of calcium, some of those anti-nutrients include oxalic acid and phytic acid, the former found primarily in vegetables, the latter in whole grains and legumes. Both have the ability to combine with minerals like calcium and iron to form an insoluble salt that the body doesn't absorb. Essentially, there is only a select group of green leafy vegetables whose calcium is highly bioavailable: broccoli, bok choy and kale. Others, like spinach, rhubarb and Swiss chard, are practically useless in terms of calcium. Milk has a fairly constant rate of bioavailability, around 32%, so that omnivores and lacto-vegetarians who consume three milk or dairy products per day are assured of meeting their calcium requirements. Those who consume only foods from the

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

plant kingdom, however, are extremely likely to be under-nourished in terms of calcium (and other nutrients as well, unless they take supplements: Reddy and Saunders, 1990).

Calcium is most heralded, of course, for its role in bone growth and maintenance. Some of the confusion over the need for calcium arises from certain cultures' low calcium intake coupled with a seemingly low incidence of osteoporosis. The argument is flawed on several counts: first, while a culture might not have a dairy-consuming tradition, they may very well obtain their calcium from other sources, like small fish with their bones or vegetables with a high level of bioavailability. Second, in developing countries people quite often don't live long enough to develop osteoporosis; third, records of osteoporosis in such countries are very often lacking; and fourth, vitamin D via sunlight exposure is very often at levels higher than in the so-called dairying countries of the northern hemisphere. Osteoporosis is dependent on factors other than calcium intake. Genetics, hormone status, and weight-bearing exercise all have a determining role in the status of one's bone health. An excellent study (Tang *et al.*, 2008) reviewed calcium and its ability to prevent osteoporotic fractures in people over 50. Of course, bone health is but one of the functions of calcium in the body. While roughly 2% of the average adult's total body weight is calcium, and nearly all of that is found in bones and teeth, the rest is performing functions that are no less important. Among other things, calcium plays a vital role in blood clotting: after an injury that results in a cut, calcium enables platelets to release thromboplastin which in turn activates the prothrombin needed to make thrombin which then converts fibrinogen to fibrin, which makes the clot that seals the wound. Calcium is also required for the absorption of dietary vitamin B12, the regulation of muscle relaxation and contraction, the creation of a neurotransmitter called acetylcholine and the activation of many crucial enzymes like pancreatic lipase. Having said all of the foregoing, what mustn't be forgotten is that calcium can't do it alone. And that brings us to some of the other important minerals available in the dairy package.

IP Address: 129.132.208.100

*Potassium, magnesium, phosphorus and zinc*

Potassium is an interesting mineral (well, dietitians and nutritionists find it interesting) for a couple of reasons. First of all, although it is commonly associated with sodium, in contrast to sodium it is concentrated within the cells. Nerve and muscle cells are especially rich in potassium. The main functions of potassium are the same as those of sodium: maintenance of fluid balance and volume. But it also has a role in carbohydrate metabolism, enhancement of protein synthesis and muscle contraction and nerve impulse conduction. This might not be of particular interest to the average person, but is of great interest to those who want to reduce the risk of developing high blood pressure – and that's where having a good amount of potassium in the diet comes into play. We have strong evidence that the interplay between calcium, potassium and magnesium has a tremendous impact on blood pressure (Appel *et al.*, 1997).

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

That brings us to another interesting fact about potassium – where you find it. It is fairly routine to note that whenever a patient is asked about what foods they would choose for potassium they invariably answer bananas and oranges. They are right: those are excellent sources, but they frequently ignore three equally good, if not better sources: yoghurt, milk and baked potato with the skin.

There's actually a third interesting thing about potassium, one that wasn't well known until recently, which is the role that this mineral plays in reducing the risk of osteoporosis. A number of recent studies (Ilich and Kerstetter, 2000) have shown that potassium is associated with increased bone mass in adults. We're not exactly sure what the connection is, but it's thought to be due to the way in which potassium reduces the loss of calcium in the urine.

It is extremely interesting that one food, milk, should contain as a package the many nutrients necessary for bone health. Yes, milk contains calcium, and yes, it is rich in potassium, and then comes magnesium. In fact, next to liver, milk, cheese and yoghurt are about the best sources of magnesium that one can find. The village of Epsom in England was where people first noticed that drinking from a pool of bitter-tasting water made them feel healthily purified. Later in the seventeenth century, somebody crystallized its salts and marketed 'Epsom Salts' to great acclaim. It wasn't until the nineteenth century that magnesium was identified as the key ingredient in the salt and it became well known for its use in photographic flashes and flares, and more recently as a metal valued for its lightness in space-age technology. But what does magnesium have to do with one's health? Among the many functions of magnesium is its role in glucose and fatty acid metabolism, amino acid activation, nervous activity and muscle contraction. Of all its jobs, however, none is more important than its role in bone metabolism. This is perhaps as good an explanation as any of the inappropriateness or maybe better said, inefficiency, of adding calcium to various beverages: all people will get is the added calcium and, as we know, healthy bones and a healthy body depend on a whole toolkit of minerals and vitamins, not just calcium. Of commonly eaten foods, in the portions usually consumed, dairy products are the best sources of magnesium, followed (in order of decreasing content) by breads and cereals, vegetables, meats and poultry, and fruits. Magnesium is to plants what iron is to animals. Just as iron is the 'core' atom of haemoglobin, magnesium is the 'core' atom of chlorophyll, the green pigment that enables plants, in the presence of light, to transform carbon dioxide and water into carbohydrates. It thus has some claim to being, next to carbon, the element most important to life. And dairy products are among the best sources of it.

If the average adult were to consume three servings (250 mL each) of milk per day, they would give themselves roughly one-third of their daily requirement for zinc. Since milk's zinc is for the most part tied up with its protein, with very little in the lipid fraction, the presence or absence of fat in the chosen milk has little bearing on its zinc content. Not only is zinc necessary for growth and development, it is also crucial for wound healing and enhanced immune status. Cultures that are deprived of milk products and consume foods rich in

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

unleavened whole-grain products (and thus, phytic acid) have been seen to experience zinc deficiency, especially Egypt and Iran in the early 1960s. Delayed growth and maturation, especially sexual maturation, are the major symptoms (Sandstead, 1968).

Phosphorus is one of those nutrients which, in the words of American comedian Rodney Dangerfield, 'gets no respect'. This is possibly due to the ongoing controversy about the impact of dietary phosphorus and especially the ratio of calcium to phosphorus and its relationship to bone health. There's little doubt that the increased consumption of phosphate salts as an additive in various food products and colas (Institute of Medicine, 1997a) has seen many adults exceeding their recommended intakes; the question is whether or not that level is deleterious to human bone. While it's been argued that a ratio of 4:1 phosphorus : calcium is indeed harmful (Calvo and Park, 1996; Kemi *et al.*, 2006), the high calcium content of milk (along with a high phosphorus content) renders its ratio of phosphorus to calcium in the order of 0.8:1. Aside from its role in bone health, phosphorus is also a key player in metabolism.

### 1.3.3 Vitamins in milk

*Water-soluble vitamins in milk*

It would be hard to come up with a vitamin that hasn't been found in milk. Even the water-soluble vitamin thiamin, long dismissed as not abundant in milk and mostly destroyed by the pasteurization process, provides through three servings (250 mL) per day about a third of the average adult's requirements. Thiamin is crucial to the energy-generating reactions involving carbohydrates, fatty acids and amino acids. Riboflavin, the most important vitamin in milk's armament, combines with proteins to form flavoproteins that participate in the energy-producing reactions of the cell. Three servings of milk will easily put most adults over the top regarding their riboflavin requirements. Niacin is another of milk's water-soluble vitamins – and not to be taken lightly. While similar to riboflavin in its various functions, niacin promotes health of both mind and body. The amino acid tryptophan is also present in milk and can be synthesized into niacin. The combination of niacin and tryptophan found in three servings of milk will supply roughly 65% of the average adult's needs. Milk is also a good source of pantothenic acid, folic acid, pyridoxine and biotin, and three servings would provide all of one's need for vitamin B12 (Institute of Medicine, 1998). About the only vitamin that doesn't show up in significant amounts in milk is ascorbic acid (Institute of Medicine, 2000).

*Fat-soluble vitamins in milk*

Milk contains each of the fat-soluble vitamins, but vitamin A and its precursors, the carotenoids, are present in the most impressive amounts. Because of its fat solubility, this vitamin is found in the fat portion of milk and therefore is added to fat-reduced and fat-free milks at least to the level that the whole product would contain. There is currently some controversy surrounding the effect of

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

vitamin A on bone health (Anderson, 2002), but there is no doubt about the overall need for vitamin A in the diet. While excess vitamin A, especially through supplements, might be harmful to bones, one study (Promislow *et al.*, 2002) indicated bone problems when vitamin A was both deficient and excessive. Even three servings of whole milk per day supplies only about one quarter of an adult's needs, so while milk is an important source of the nutrient, it stretches credulity to think that its vitamin A content would pose a threat to bone health.

Milk as it comes straight from the cow is not a great source of vitamin D (Jensen, 1995). This, coupled with the fact that a vitamin D deficiency in children leads to rickets (and osteomalacia in adults), has led many, though not all, governments in the western world to mandate the addition of vitamin D to fluid milk, usually at the 100 IU (International Unit) per serving level. Much research has been conducted on the importance of vitamin D (Vieth, 1999), but suffice it to say that the addition of vitamin D to milk is of extreme importance. Not only does vitamin D enhance the absorption of calcium (Institute of Medicine, 1997a), but as we shall discuss later its deficiency has been implicated in a number of diseases from multiple sclerosis to depression to breast cancer. Vitamin E exists in relatively small amounts in milk, but as an antioxidant perhaps serves to spare the cells from oxidative damage (Fox, 1997). Likewise, vitamin K is not extremely well represented in fluid milk, but might have an as yet undefined role in bone health (Institute of Medicine, 1997b).

IP Address: 129.132.208.100

## 1.4 Disadvantages of a low-dairy diet

The health repercussions of avoiding milk and milk products are not limited to an increased risk of osteoporosis. While it's true that calcium is the only nutrient whose requirements are difficult to meet without dairy (aside from vitamin D in the absence of sun exposure), milk is unique in that it offers such a wide range of nutrients. In a nutshell, people who consume the equivalent of three servings of dairy on a daily basis are likely to have a lower risk of obesity, type 2 diabetes, hypertension, colon cancer, breast cancer, premenstrual syndrome, and kidney stones.

### 1.4.1 Obesity

Obesity, despite the popular notion, is not a recent phenomenon. Unfortunately, one of the first things that a dieter does upon starting a weight-loss programme is to reduce or abandon the intake of milk and milk products under the mistaken impression that such foods are 'fattening'. Equally unfortunate is the fact that such behaviour most likely will lead to the dieter ending up with less bone matter than they had to start with. The evidence now, however, indicates that not only is dairy not 'fattening', the very opposite is most likely closer to reality. Research (Zemel *et al.*, 2000) drew attention to an intriguing effect of diets rich

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

in calcium, namely their ability to promote body fat loss. In a cross-sectional human epidemiological study, the risk of being in the highest quartile of body fat was reduced by increasing calcium intake and dairy consumption in women and men. Another study (Zemel, 2003) proposed a mechanism and provided evidence for this mechanism derived from a combination of *in vitro* human cell experiments and animal feeding trials. In fact, the high dairy diet was more effective than the high calcium-supplement diet at reducing body weight gain, decreasing fatty acid synthesis activity, stimulating lipolysis and decreasing abdominal fat pad mass when calorie intake was similarly reduced. Other researchers (Heaney *et al.*, 2002), have reviewed the literature and reached similar conclusions. An inverse relationship was also found (Mirmiran *et al.*, 2005) between dairy consumption and body mass index. The evidence that calcium/dairy intake impacts weight loss far 'outweighs' the evidence of no effect, including that published in the *American Journal of Clinical Nutrition* (Parikh and Yanovski, 2003). Studies on children (Carruth and Skinner, 2001) have delivered similar findings. Moreover, given that energy-reduced diets may cause bone loss, along with weight management problems (Major *et al.*, 2008), it is especially important that weight-loss diets don't deprive the dieter of the recommended servings of dairy products.

### 1.4.2 Type 2 diabetes/metabolic syndrome

Aside from the likelihood of calcium insufficiency increasing the risk of obesity, there are other increased risks for chronic illness that accompanies a dairy-deprived diet. Reduced calcium intake has been associated with hypertension (McCarron *et al.*, 1984; Hamet, 1995; Appel *et al.*, 1997), type 2 diabetes (Choi *et al.*, 2005a; Pittas *et al.*, 2006), cholesterol concentrations (Jacqmain *et al.*, 2003), and the clustering of these conditions to form what is known as metabolic syndrome (Pereira *et al.*, 2002). Also referred to as the insulin resistance syndrome and syndrome x, it has long been thought that a combination of obesity, hyperinsulinaemia and insulin resistance leads to glucose intolerance, low HDL levels and elevated triglycerides, but the role played by diet in the development of the syndrome has not been fully explained. Pereira and his group found that overweight individuals who consumed the most dairy actually had a significantly lower risk of future incidence of metabolic syndrome. Studying a group of Iranians (Azadbakht *et al.*, 2005) researchers found an inverse relationship between dairy consumption and metabolic syndrome, as did a group (Elwood *et al.*, 2007) looking at subjects in Wales. The syndrome is on the rise in younger people, just as milk and dairy consumption is declining and intake of soft drinks and refined carbohydrates is on the upswing. More recently (Lutsey *et al.*, 2008) found that a 'western' type diet (fried foods, meats, diet sodas) were strongly associated with metabolic syndrome, while dairy consumption provided protection.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

### 1.4.3 Hypertension/stroke

Reference has already been made to the DASH diet (in which a drop in blood pressure brought about by inclusion of 8–10 servings of fruits and vegetables was doubled when the diet included three servings of dairy). Meta-analyses of the epidemiological evidence and of the results of randomized controlled trials have concluded that increased calcium intake is associated with a reduction in both systolic and diastolic blood pressure (Birkett, 1998; Griffith *et al.*, 1999). There has been much additional research (Massey, 2001; Wang *et al.*, 2008) indicating more dairy, lower blood pressure. Similarly, other research (Ruidavets *et al.*, 2006; Daniel, 2006) has found an important impact of dairy products on blood pressure and cardiovascular disease (Elwood *et al.*, 2005; Umesawa *et al.*, 2006). This is most likely due not just to dairy's calcium content, but rather to the combination of calcium, potassium and magnesium that is part and parcel of the dairy package, not to mention the potential role of milk protein in exerting an anti-hypertensive effect (Townsend *et al.*, 2004). A study in Japan (Kinjo *et al.*, 1999), where cerebrovascular disease is one of the major health concerns, found a possible protective effect of milk, meat and fish. Another interesting proposal has been put forth (Metz *et al.*, 1999) in a study that found a relationship between blood pressure, calcium intake and bone density: blood pressure varied indirectly with bone mass and density.

### 1.4.4 Cancer

IP Address: 129.132.208.100

From the superficially amusing notion that pizza protects against cancer to studies in Japan showing reduced risk of bladder cancer and breast cancer in dairy consumers, there is increasing evidence that something in milk and milk products offers protection against various forms of the disease. The pizza study (Gallus *et al.*, 2003) compared the diets of 3000 cancer sufferers with 5000 non-cancer patients and found that the risk of oral, oesophageal and colon cancer fell by 34, 59 and 26%, respectively. The authors speculated that tomato sauce was responsible, but the pizzas consumed contained as much mozzarella cheese as tomato sauce. From the perceived ridiculous to the sublime, a study (Wakai *et al.*, 2000) in Japan (where popular misconception has it that dairy is seldom consumed) found that the greater the intake of milk (and of saturated fatty acids), the lower the odds ratio for bladder cancer. Again in Japan, researchers (Hirose *et al.*, 2003) found a reduced risk of breast cancer in pre-and post-menopausal women with a high level of milk consumption. Breast cancer and diet was also studied in Finland (Knekt *et al.*, 1996) where it was found that women who consumed the most milk had less than half the risk of breast cancer compared to women consuming the least milk. Also in Finland (Aro *et al.*, 2000) researchers found an inverse association between dietary and serum conjugated linoleic acid (present in the fat of ruminant animals) and the risk of breast cancer in post-menopausal women. A study in Norway (Hjartåker *et al.*, 2001) showed that childhood and adult milk consumption could protect against breast cancer. Women drinking more than three glasses of milk per day had half the risk of

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

breast cancer compared to women not drinking milk. A negative association between skim and low-fat milk and pre-menopausal breast cancer has also been reported (Shin *et al.*, 2002).

Of all the research examining the relationship between dairy consumption and cancer risk-reduction, perhaps the most persuasive is that regarding colon cancer. At least 30 years ago researchers (Phillips, 1975) found an inverse association between milk consumption and colon cancer risk. Looking at dietary and supplemental calcium (Hyman *et al.*, 1998), investigators found a high calcium intake possibly associated with a reduced risk of recurring colorectal adenomas, while another group (Wu *et al.*, 2002) found an association between a higher calcium intake and decreased risk of distal colon cancer. A pooled analysis of 10 cohort studies (Cho *et al.*, 2004) revealed higher consumption of milk and calcium to be associated with a lower risk of colorectal cancer, while a prospective study of the Swedish mammography cohort (Larsson *et al.*, 2005) suggested that high intakes of high-fat dairy foods and conjugated linoleic acid might afford protection from that same cancer. A similar prospective study of Swedish men (Larsson *et al.*, 2006) supported the idea of an inverse association between intakes of dairy foods and calcium and the risk of colorectal cancer. The anticancer potential of cow's milk is addressed comprehensively in a 2000 study (Gill and Cross, 2000).

### 1.4.5 Other possible risks associated with a low-dairy diet

IP Address: 129.132.208.100

Both premenstrual syndrome (PMS) and gout are frequently the butt of jokes and some amusement among those not suffering from either condition, but to the afflicted they are no laughing matter. Interestingly enough, both ailments have been found in greater numbers in people whose dairy intake is in the low to negligible category. Several studies (Thys-Jacobs *et al.*, 1998; Thys-Jacobs, 2000) have indicated that higher intakes of dairy products (and their constituent minerals) resulted in a decrease of PMS symptoms, and another study (Bertone-Johnson *et al.*, 2005) showed that when the nutrients calcium and vitamin D were consumed in food (roughly four servings per day), their impact on PMS symptoms was significantly greater than when the food equivalent was one serving per day or less. Researchers looking at the relationship between diet and gout (Choi *et al.*, 2004) found a substantially decreased risk of gout in men who had a higher level of dairy product consumption, and the same lead investigator (Choi *et al.*, 2005b) found an inverse association between dairy intake and uric acid levels. Perhaps even more interesting is an article (Johnson and Rideout, 2004) pointing out a connection between high uric acid levels, gout and cardiovascular disease. An article (Fang and Alderman, 2000) found elevated serum uric acid levels to be a predictor of cardiovascular mortality. Given the evidence that higher intakes of dairy reduce the risk of gout and of CHD, it would seem to follow that the relationships are more than merely coincidental.

Much less the subject of jocularity, kidney stones and their passing have long been attributed to a diet rich in calcium. That's been shown to be at least partly

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

correct – if the dietary calcium is accompanied by oxalic acid. Foods rich in both those substances, spinach and rhubarb to name a couple, are definitely *verboten*. However, contrary to what many people believe – including many health professionals – diets rich in dairy calcium actually help prevent the occurrence and recurrence of kidney stones. Several studies (Stern, 1993; Curhan *et al.*, 1993; Curhan, 1997) have demonstrated that dairy calcium and protein will reduce the likelihood of kidney stone formation. Conversely, diets low in dairy put vulnerable patients at greater risk of developing stones.

Another group of individuals for whom a diet low in dairy is apt to spell trouble is pregnant women. No less an august body than the World Health Organization (Villar *et al.*, 2006) has concluded that pregnant women globally are failing to consume the recommended daily supply of 1200 mg of calcium; dairy products are the easiest and most reliable source of the mineral. Among the complications likely to befall the under-consumer are gestational hypertension, eclampsia, maternal morbidity and mortality, and pre-term delivery. Topping it all off is a study (McCarron and Heaney, 2004) estimating the savings in health-care dollars to be gained by the simple adherence to the dictum of three servings of dairy per day. Implicit in that, of course, is an improved quality of life thanks to a reduced risk of so many chronic illnesses.

## 1.5 Sources of further information and advice

IP Address: 129.132.208.100

The foregoing is but a sample of the abundant evidence of the role and importance of milk and dairy in the diet. For those interested in a complete examination of all things related to nutrition and dairy, there are several resources that are invaluable. The first of these that I would bring to your attention is *Handbook of Dairy Foods and Nutrition*, 3rd edition, by Gregory D. Miller, Judith K. Jarvis and Lois D. McBean (CRC Press). This book not only addresses the nutritional content and benefits of milk and milk products, but also delivers relevant information on the relationship between dairy consumption and various chronic conditions that plague us globally. Another excellent reference is *Dairy Nutrition & Health* published by the Dairy Council of the United Kingdom. Specific issues examined include lactose intolerance, cow's milk allergy and dental health, along with research on topics like colon cancer and calcium bioavailability. Those interested in obtaining the book can email info@dairycouncil.org.uk. To these resources I would add anything written by Peter Parodi and Robert Heaney, true experts on the subject of dairy's role in nutrition.

Because much of the misunderstanding about dairy's role in the diet centres on its fat component, a paper that is a 'must read' is by Bill Lands, titled 'A critique of paradoxes in current advice on dietary lipids', published in the March 2008 issue of *Progress in Lipid Research*.

To sum up, and using phraseology that is somewhat inelegant but nonetheless honest, in the view of this nutritionist anyone who doesn't have three servings of dairy each day is, quite simply, nuts.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

## 1.6 References

ABBOTT R D, CURB J D, RODRIGUEZ B I, SHARP D S, BURCHFIEL C M and YANO K (1996), 'Effect of dietary calcium and milk consumption on risk of thromboembolic stroke in older middle-aged men. The Honolulu Heart Program', *Stroke*, 217, 813–818.

ANDERSON J J B (2002), 'Oversupplementation of vitamin A and osteoporotic fractures in the elderly: to supplement or not to supplement', *J Bone Min Res*, 17, 1359–1362.

APPEL L J, MOORE T J, OBARZANEK E, VOLLMER W M, SVETKEY L P, SACKS F M, BRAY G A, VOGT T M, CUTLER J A, WINDHAUSER M M, LIN P H and KARANJA N (1997), 'A clinical trial of the effects of dietary patterns on blood pressure', *N E J Med*, 336, 1117–1124.

ARO A, MÄNNISTÖ S, OVASKALNEN M, KATAJA V and UUSITUPA M (2000), 'Inverse association between dietary and serum conjugated linoleic acid and risk of breast cancer in postmenopausal women', *Nutr and Cancer*, 38(2), 151–167.

ASCHERIO A, HENNEKENS C H, BURING J E, MASTER C, STAMPFER M J and WILLETT W C (1994), 'Trans-fatty acids intake and risk of myocardial infarction', *Circ*, 89, 94–101.

ASCHERIO A, RIMM B, GIOVANNUCCI E, SPIEGELMAN D, STAMPFER M and WILLETT W C (1996), 'Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States', *Br Med J*, 313, 84–90.

AUSTIN M, BRESLOW J, HENNEKENS C, BURING J, WILLETT W C and KRAUSS R (1988), 'Low-density lipoprotein subclass patterns and risk of myocardial infarction', *JAMA,* 260(13), 1910–1916.

AZADBAKHT L, MIRMIRAN P, ESMAILLZADEH A and AZIZI F (2005), 'Dairy consumption is inversely associated with the prevalence of the metabolic syndrome in Tehranian adults', *A J Clin Nutr*, 82, 523–530.

BAL DIT SOLLIER C, DROUET L, PIGNAUD G, CHEVALLIER C, CAEN J, FIAT A M, IZQUIERDO C and JOLLES P (1996), 'Effect of kappa-casein split peptides on platelet aggregation and on thrombus formation in the guinea-pig', *Thromb Res*, 81, 427–437.

BELURY M (2002), 'Inhibition of carcinogenesis by conjugated linoleic acid: potential mechanisms of action', *J Nutr*, 132, 2995–2998.

BERTONE-JOHNSON E R, HANKINSON S E, BENDICH A, JOHNSON S R, WILLETT W C and MANSON J E (2005), 'Calcium and vitamin D intake and risk of incident premenstrual syndrome', *Arch Int Med*, 165, 1246–1252.

BIONG A, REBNORD M, FIMREITE R, TRYGG K, RINGSTAD J, THELLE D and PEDERSEN J (2008), 'Intake of dairy fat and dairy products and risk of myocardial infarction: a case-control study', *Intl J Food Sci Nutr*, 59(2), 155–165.

BIRKETT N J (1998), 'Comments on a meta-analysis of the relationship between dietary calcium intake and blood pressure', *Am J Epidem*, 148, 223–228.

BOWEN J, NOAKES M and CLIFTON P M (2004), 'A high dairy protein, high-calcium diet minimizes bone turnover in overweight adults during weight loss', *J Nutr*, 134, 568–573.

BREWER J L, BLAKE A J, RANKIN S A and DOUGLASS L W (1999), 'Theory of reasoned action predicts milk consumption in women', *JADA*, 99, 39–44.

CAEN J P, JOLLES P, BAL DIT SOLLIER C, FIAT A-M, MAZOYER E and DROUT L (1992), 'Anti-thrombotic activity of milk protein peptide sequences', *Cahiers de Nutrition et de Diététique*, 27, 33–35.

CALVO M S and PARK Y K (1996), 'Changing phosphorus content of the U.S. diet: potential for adverse effects on bone', *J Nutr*, 126(4), 1168–1180.

CARRUTH B R and SKINNER J D (2001), 'The role of dietary calcium and other nutrients in moderating body fat in preschool children', *Int J Obes Rel Metab Disord*, 25, 559–566.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

CHO E, SMITH-WARNER S A, SPIEGELMAN D, BEESON W L, VAN DE BRANDT P A, COLDITZ G A, FOLSOM A R, FRASER G E, FREUDENHEIM J L, GIOVANNUCCI E, GOLDBOHM R A, GRAHAM S, MILLER A B, PIETINEN P, POTTER J D, ROHAN T E, TERRY P, TONIOLO P, VIRTANEN M J, WILLETT W C, WOLK A, WU K, YAUN S S, ZELENIUCH-JACQUOTTE A and HUNTER J (2004), 'Dairy foods, calcium, and colorectal cancer: a pooled analysis of 10 cohort studies', *J Nat Can Inst*, 96, 1015–1022.

CHOI H K, ATKINSON K, KARLSON E W, WILLETT W C and CURHAN G (2004), 'Purine-rich foods, dairy and protein intake, and the risk of gout in men', *N E J Med*, 350, 1093–1103.

CHOI H K, WILLETT W C, STAMPFER M J, RIMM E and HU F B (2005a), 'Dairy consumption and risk of type 2 diabetes mellitus in men: a prospective study', *Arch Intern Med*, 165, 997–1003.

CHOI H K, LIU S and CURHAN G (2005b), 'Intake of purine-rich foods, protein, and dairy products and relationship to serum levels of uric acid: the third national health and nutrition examination survey', *Arth Rheum*, 52(1), 283–289.

CURHAN G C (1997), 'Dietary calcium, dietary protein and kidney stone formation', *Min Elect Metab*, 23, 261–264.

CURHAN G C, WILLETT W C, RIMM E B and STAMPFER M J (1993), 'A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones', *N E J Med*, 328(12), 833–838.

DAIRY COUNCIL OF THE UNITED KINGDOM (2007), *Dairy Nutrition and Health*, London, The Dairy Council.

DANIEL E E (2006), 'Intake of dairy products on calcium and blood pressure'. *J Hyper*, 24, 633–634.

DIAMOND H and DIAMOND M (1987), *Living Health*, New York, Warner Books.

DILLEHAY D L, WEBB S K, SCHMELZ E-M and MERRILL A H (1994), 'Dietary sphingomyelin inhibits 1,2-dimethylhydrazine-induced colon cancer in CF1 mice', *J Nutr*, 124(5), 615–620.

DREON D M, FERNSTROM H, MILLER B and KRAUSS R M (1994), 'Low-density lipoprotein subclass patterns and lipoprotein response to a reduced-fat diet in men', *FASEB*, 8, 121–126.

DUYFF R L (2006), *American Dietetic Association Complete Food and Nutrition Guide*, Hoboken, NJ, John Wiley & Sons.

ELWOOD P C, STRAIN J J, ROBSON P J, FEHILY A M, HUGHES J, PICKERING J and NESS A (2005), 'Milk consumption, stroke, and heart attack risk: evidence from the Caerphilly cohort of older men', *JECH*, 59, 502–505.

ELWOOD P C, PICKERING J E and FEHILY A M (2007), 'Milk and dairy consumption, diabetes and the metabolic syndrome: the Caerphilly prospective study', *JECH*, 61, 695–698.

FANG J and ALDERMAN M H (2000), 'Serum uric acid and cardiovascular mortality: the NHANES I epidemiologic follow-up study, 1971–1992', *JAMA*, 283(18), 2404–2410.

FEHILY A M, YARNELL J W G, SWEETNAM P M and ELWOOD P C (1993), 'Diet and incident ischaemic heart disease: the Caerphilly Study'. *B J Nutr*, 69, 303–314.

FESKANICH D, WILLETT W C, STAMPFER M J and COLDITZ G A (1996), 'Protein consumption and bone fractures in women', *Am J Epidem*, 143(5), 472–479.

FITZGERALD R J, MURRAY B A and WALSH D J (2004), 'Hypotensive peptides from milk proteins', *J Nutr*, 134, 980S–988S.

FOX P F, ED. (1997), *Advanced Dairy Chemistry, Volume 3. Lactose, Water Salts and Vitamins*, 2nd edn, New York, Chapman & Hall.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

GALLUS S, BOSETTI C, NEGRI E, TALAMINI R, MONTELLA M, CONTI E, FRANCESCHI S and LA VECCHIA C (2003), 'Does pizza protect against cancer?', *Intl J Cancer*, 107, 283–284.

GARTSIDE P S, WANG P and GLUECK C J (1998), 'Prospective assessment of coronary heart disease risk factors: the NHANES I epidemiologic follow-up study (NHEFS) 16-year follow-up', *J A Coll Nutr*, 17(3), 263–269.

GILL H S and CROSS M L (2000), 'Anticancer properties of bovine milk', *B J Nutr*, 84, S161–S166.

GILLMAN M W, CUPPLES L A, MILLEN B E, ELLISON R C and WOLF P A (1997a), 'Inverse association of dietary fat with development of ischemic stroke in men', *JAMA*, 278, 2145–2150.

GILLMAN M W, CUPPLES A L, GAGNON D, MILLEN B E, ELLISON R C and CASTELLI W P (1997b), 'Margarine intake and subsequent coronary heart disease in men', *Epidem*, 8, 144–149.

GRIFFITH L E, GUYATT G H, COOK R J, BUCHER H C and COOK D J (1999), 'The influence of dietary and non-dietary calcium supplementation on blood pressure: an updated meta-analysis of randomized controlled trials', *A J Hyper*, 12, 84–92.

GULLIVER P and HORWATH C (2001), 'Women's readiness to follow milk product consumption recommendations: design and evaluation of a ''stage of change'' algorithm', *J Hum Nutr Diet*, 14(4), 277–286.

HAGUE A and PARASKEVA C (1995), 'The short-chain fatty acid butyrate induces apoptosis in colorectal tumor cell lines', *Euro J Cancer Prev*, 4(5), 359–364.

HAMET P (1995), 'The evaluation of the scientific evidence for a relationship between calcium and hypertension', *J Nutr*, 125, s311–s317.

HARRIS M (1986), *Good to Eat: Riddles of Food and Culture*, New York, Simon and Schuster.

HEANEY R P (1998), 'Excess dietary protein may not adversely affect bone', *J Nutr*, 128(6), 1054–1057.

HEANEY R P (2000), 'Calcium, dairy products and osteoporosis', *J A Coll Nutr*, 19(2), 83S–99S.

HEANEY R P, DAVIES K M and BARGER-LUX M J (2002), 'Calcium and weight: clinical studies', *J A Coll Nutr*, 21(2), 152S–155S.

HIROSE K, TAKEZAKI T, HAMAJIMA N, MIURA S and TAJIMA K (2003), 'Dietary factors protective against breast cancer in Japanese premenopausal and postmenopausal women', *Inl J Cancer*, 107, 276–282.

HJARTÅKER A, LAAKE P and LUND E (2001), 'Childhood and adult milk consumption and risk of premenopausal breast cancer in a cohort of 48,844 women – the Norwegian women and cancer study', *Int J Cancer*, 93(6), 888–893.

HOLT P R (1999), 'Dairy foods and prevention of colon cancer: human studies', *J Am Coll Nutr*, 18(5), 379S–381S.

HU F B and WILLETT W (2000), letter to the editor, 'Reply to O H Holmqvist', *Am J Clin Nutr*, 71(3), 848–849.

HYMAN J, BARON J A, DAIN B J, SANDLER R S, HAILE R W, MANDEL J S, MOTT L A and GREENBERG E R (1998), 'Dietary and supplementary calcium and the recurrence of colorectal adenomas', *Cancer Epidem Bio Prev*, 7, 291–295.

ILICH J Z and KERSTETTER J E (2000), 'Nutrition in bone health revisited', *J Am Coll Nutr,* 19(6), 715–737.

INSTITUTE OF MEDICINE (1997a), *Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D and fluoride*, Washington, DC, National Academy Press.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

INSTITUTE OF MEDICINE (1997b), *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc*, Washington, DC, National Academy Press.

INSTITUTE OF MEDICINE (1998), *Dietary reference intakes for thiamine, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin and choline*, Washington, DC, National Academy Press.

INSTITUTE OF MEDICINE (2000), *Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids*, Washington, DC, National Academy Press.

JACQMAIN M, DOUCET E, DESPRÉS J-P, BOUCHARD C and TREMBLAY A (2003), 'Calcium intake, body composition, and lipoprotein-lipid concentration in adults', *Am J Clin Nutr*, 77(6), 1448–1452.

JENSEN R G, ed. (1995), *Handbook of milk composition*, New York, Academic Press.

JOHNSON R J and RIDEOUT D V M (2004), 'Uric acid and diet – insights into the epidemic of cardiovascular disease', *N E J Med*, 350(11), 1071–1072.

KAESS B, FISCHER M, BAESSLER A, STARK K, HUBER F, KREMER W, KALBITZER H R, SCHUNKERT H, RIEGGER G and HENGSTENBERG C (2008), 'The lipoprotein subfraction profile: heritability and identification of quantitative trait loci', *J Lip Res*, 49, 715–723.

KEMI V E, KARKKAINEN M U M and LAMBERG-ALLARDT C J E (2006), 'High phosphorus intakes acutely and negatively affect Ca and bone metabolism in a dose-dependent manner in healthy young females', *Br J Nutr*, 96(3), 545–552.

KINJO Y, BERAL V, AKIBA S, KEY T, MIZUNO S, APPLEBY P, YAMAGUCHI N, WATANABE S and DOLL R (1999), 'Possible protective effect of milk, meat and fish for cerebrovascular disease mortality in Japan', *J Epidemiol*, 9, 268–274.

KNEKT P, JÄRVINEN R, SEPPÄNEN R, PUKKALA E and AROMAA A (1996), 'Intake of dairy products and risk of breast cancer', *B J Cancer*, 73, 687–691.

KONTOGIANNI M D, PANAIOTAKOS D B, CHRYSOHOOU C, PITSAVOS C and STEFANADIS C (2006), 'Modelling dairy intake on the development of acute coronary syndromes: the CARDIO 2000 study', *E J Card Prev Rehab*, 13(5), 791–797.

KRAUSS M (2001), 'Dietary and genetic effects on low-density lipoprotein heterogeneity', *Ann Rev Nutr*, 21, 283–295.

KRAUSS M (2005), 'Dietary and genetic probes of atherogenic dyslipidemia', *Artero Thromb Vasc Bio*, 25, 2265–2272.

LANDS B (2008), 'A critique of paradoxes in current advice on dietary lipids', *Prog Lip Res*, 47, 77–106.

LARSON N, STORY M, WALL M and NEUMARK-SZTAINER D (2003), 'Calcium and dairy intakes of adolescents are associated with their home environment, taste preferences, personal health beliefs, and meal patterns', *JADA*, 106(11), 1816–1824.

LARSSON S C, BERGKVIST L and WOLK A (2005), 'High-fat dairy food and conjugated linoleic acid intakes in relation to colorectal cancer incidence in the Swedish Mammography Cohort', *A J Clin Nutr*, 82, 894–900.

LARSSON S C, BERGKVIST L, RUTEGÅRD J, GIOVANNUCCI E and WOLK A (2006), 'Calcium and dairy food intakes are inversely associated with colorectal cancer risk in the Cohort of Swedish Men', *A J Clin Nutr*, 83, 667–673.

LUTSEY P L, STEFFEN L M and STEVENS J (2008), 'Dietary intake and the development of the metabolic syndrome', *Circ*, 117, 754–761.

MAJOR G C, CHAPUT J-P, LEDOUX M, ST-PIERRE S, ANDERSON G H, ZEMEL M B and TREMBLAY A (2008), 'Recent developments in calcium-related obesity research', *Ob Rev*, 9(5), 428–445.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

MASSEY L K (2001), 'Dairy food consumption, blood pressure and stroke', *J Nutr*, 131, 1875–1878.

MCCARRON D A and HEANEY R P (2004), 'Estimated healthcare savings associated with adequate dairy food intake', *Am J Hypertens*, 17, 88–97.

MCCARRON D A, MORRIS C D and STANTON J L (1984), 'Hypertension and calcium', *Science*, 226, 386–393.

MCGEE H (1984), 'Milk and dairy products', in McGee H, *On Food and Cooking: The Science and Lore of the Kitchen*, New York, Scribner, 3–53.

MENSINK R P, ZOCK P L, KESTER A D and KATAN M B (2003), 'Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials', *Am J Clin Nutr*, 77(5), 1146–1155.

METZ J A, MORRIS C D, ROBERTS L A, MCCLUNG M R and MCCARRON D A (1999), 'Blood pressure and calcium intake are related to bone density in adult males', *B J Nutr*, 81, 383–388.

MILLER G D, JARVIS J K and MCBEAN L D (2007), *Handbook of Dairy Foods and Nutrition*, 3rd edn, Boca Raton, FL, CRC Press.

MIRMIRAN P, ESMAILLZADEH A and AZIZI F (2005), 'Dairy consumption and body mass index: an inverse relationship', *Int J ObRel Metab Disord*, 29, 115–121.

MORRIS D (1994), *The Human Animal: A Personal View of the Human Species*, London, BBC Books.

MOZAFFARIAN D, RIMM B and HERRINGTON D M (2004), 'Dietary fats, carbohydrate, and progression of coronary atherosclerosis in postmenopausal women', *A J Clin Nutr*, 80, 1175–1184.

MUNRO H N and CRIM M C (1988), 'The protein and amino acids', in Shills M E and Young V R (eds), *Modern Nutrition in Health and Disease*, 7th edn, Philadelphia, PA, Lea & Febiger, 1–37.

NESS A R, SMITH G D and HART C (2001), 'Milk, coronary heart disease and mortality', *JECH*, 55, 379–382.

NEWMARK H L and LIPKIN M (1992), 'Calcium, vitamin D and colon cancer', *Cancer Res*, 52, 2067s–2070s.

PARIKH S J and YANOVSKI J A (2003), 'Calcium intake and adiposity', *A J Clin Nutr*, 77, 281–287.

PARODI P W (1997), 'Cow's milk fat components as potential anticarcinogenic agents', *J Nutr*, 127, 1055–1060.

PARODI P W (2001), 'Cow's milk components with anti-cancer potential', *Aust J Dairy Technol*, 56, 65–73.

PARODI P W (2004), 'Milk fat in human nutrition', *Aust J Dairy Technol*, 59, 3–59.

PEDERSEN J I, RINGSTAD J, ALMENDINGEN K, HAUGEN T S, STENSVOLD I and THELLE D S (2000), 'Adipose tissue fatty acids and risk of myocardial infarction – a case control study', *Euro J Clin Nutr*, 54(8), 618–625.

PEREIRA M A, JACOBS D R, VAN HORN L, SLATTERY M L, KARTASHOV A I and LUDWIG D S (2002), 'Dairy consumption, obesity, and the insulin resistance syndrome in young adults. The CARDIA study', *JAMA*, 287(16), 2081–2089.

PFEUFFER M and SCHREZENMEIR J (2000), 'Bioactive substances in milk with properties decreasing risk of cardiovascular diseases', *B J Nutr*, 84, S155–S159.

PHILLIPS R L (1975), 'Role of life-style and dietary habits in risk of colon cancer among Seventh-Day Adventists', *Cancer Res*, 35, 3513–3522.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

PIETINEN P, ASCHERIO A, KORHONEN P, HARTMAN A M, WILLETT W C, ALBANES D and VIRTAMO J (1997), 'Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men: the alpha-tocopherol, beta-carotene cancer prevention study', *A J Epidem*, 145, 876–887.

PIMENTEL D (1980), *Handbook of Energy Utilization in Agriculture*, Boca Raton, FL, CRC Press.

PIMENTEL D and PIMENTEL M (2003), 'World population, food, natural resources, and survival', *World Futures*, 59, 145–167.

PITTAS A G, DAWSON-HUGHES B, LI T, VAN DAM R M, WILLETT W C, MANSON J E and HU F B (2006), 'Vitamin D and calcium intake in relation to type 2 diabetes in women', *Diab Care*, 29(3), 650–656.

PROMISLOW J H E, GOODMAN-GRUEN D, SLYMEN D J and BARRETT-CONNOR E (2002), 'Retinol intake and bone mineral density in the elderly: the Rancho Bernardo study', *J Bone Min Res*, 17(8), 1349–1358.

RAVNSKOV U (1998), 'The questionable role of saturated and polyunsaturated fatty acids in cardiovascular disease', *J Clin Epidemiol*, 51, 443–460.

REDDY S and SAUNDERS T B (1990), 'Haematological studies on pre-menopausal Indian and Caucasian vegetarians compared with Caucasian omnivores', *B J Nutr*, 64, 331–338.

RUIDAVETS J B, BONGARD V, SIMON C, DALLONGEVILLE J, DUCIMETIÈRE P, ARVEILER D, AMOUYEL P, BINGHAM A and FERRIÈRES J (2006), 'Independent contribution of dairy products and calcium intake to blood pressure variations at a population level', *J Hyper*, 24, 671–681.

RUTHERFORD K J and GILL H S (2000), 'Peptides affecting coagulation', *Br J Nutr*, 84(S1), s99–s102.

SAMUELSON G, BRATTEBY L-E, MOHSEN R and VESSBY B (2001), 'Dietary fat intake in healthy adolescents: inverse relationships between the estimated intake of saturated fatty acids and serum cholesterol', *B J Nutr*, 85, 333–341.

SANDSTEAD H H (1968), 'Zinc, a metal to grow on', *Nutr Today*, 3(1), 12–17.

SAVAIANO D A, BOUSHEY C J and MCCABE G P (2006), 'Lactose intolerance symptoms assessed by meta-analysis: a grain of truth that leads to exaggeration', *J Nutr*, 136, 1107–1113.

SCRIMSHAW N S and MURRAY E B (1988), 'The acceptability of milk and milk products in populations with a high prevalence of lactose intolerance', *Am J Clin Nutr*, 48, 1083–1085.

SHIN M-H, HOLMES M D, HANKINSON S E, WU K, COLDITZ G A and WILLETT W C (2002), 'Intake of dairy products, calcium, and vitamin D and risk of breast cancer', *J Natl Cancer Inst*, 94, 1301–1311.

SJOGREN P, ROSELL M, SKOGLUND-ANDERSON C, ZDRAVKOVIC S, VESSBY B, DEFAIRE U, HAMSTEN A, HELLENIUS M-L and FISHER R M (2004), 'Milk-derived fatty acids are associated with a more favourable LDL particle size distribution in healthy men', *J Nutr*, 134, 1729–1735.

SMEDMAN A, GUSTAFSSON I-B, BERGLUND L and VESSBY B (1999), 'Pentadecanoic acid in serum as a marker for intake of milk fat: relations between intake of milk fat and metabolic risk factors', *A J Clin Nutr*, 69, 22–29.

SMITH J G and GERMAN J B (1995), 'Molecular and genetic effects of dietary-derived butyric acid', *Food Technol*, 49(11), 87–90.

STERN R (1993), 'Calcium and kidney stones', *N E J Med* (letter), 329(7), 508–509.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

SUAREZ F L, SAVAIANO D, ARBISI P and LEVITT M D (1997), 'Tolerance to the daily ingestion of two cups of milk by individuals claiming lactose intolerance', *Am J Clin Nutr*, 65, 1502–1506.

TANG B M, ESLICK G D, NOWSON C, SMITH C and BENSOUSSAN A (2008), 'Review: Calcium supplementation, with or without vitamin D, prevents osteoporotic fractures in people >50 years of age', *A C P J Club*, 148(2), 41.

TAVANI A, GALLUS S, NEGRI E and LA VECCHIA C (2002), 'Milk, dairy products, and coronary heart disease', *JECH*, 56(6), 471–472.

THORPE M P, JACOBSON E H, LAYMAN D K, HE X, KRIS-ETHERTON P and EVANS E M (2008), 'A diet high in protein, dairy, and calcium attenuates bone loss over twelve months of weight loss and maintenance relative to a conventional high-carbohydrate diet in adults', *J Nutr*, 138, 1096–1100.

THYS-JACOBS S (2000), 'Micronutrients and the premenstrual syndrome: the case for calcium', *J Am Coll Nutr*, 19(2), 220–227.

THYS-JACOBS S, STARKEY P, BERNSTEIN D and TIAN J (1998), 'Calcium carbonate and the premenstrual syndrome: effects on premenstrual and menstrual symptoms', *Am J Obst Gyn*, 179(2), 444–452.

TOWNSEND R R, MCFADDEN C B, FORD V and CADEE J A (2004), 'A randomized, double-blind, placebo-controlled trial of casein protein hydrolysate (C 12 peptide) in human essential hypertension', *Am J Hypertens*, 17, 1056–1058.

UMESAWA M, ISO H, DATE C, YAMAMOTO A, TOYOSHIMA H, WATANABE Y, KIKUCHI S, KOIZUMI A, KONDO T, INABAB Y, TANABE N and TAMAKOSHI A (2006), 'Dietary intake of calcium in relation to mortality from cardiovascular disease (The JACC Study)', *Stroke*, 37, 20–26.

VIETH R (1999), 'Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety', *Am J Clin Nutr*, 69, 842–856.

VILLAR J, ABDEL-ALEEM H, MERIALDI M, MATHAI M, ALI M, ZAVALETA N, PURWAR J, HOFMEYR N, THI NHU NGOC N and CAMPÓDONICO L (2006), 'World Health Organization randomized trial of calcium supplementation among low calcium intake pregnant women', *Am J Obs Gyn*, 194(3), 639–649.

WAKAI K, TAKASHI M, OKAMURA K, YUBA H, SUZUKI K, MURASE T, OBATA K, ITOH H, KATO T, KOBAYASHI M, SAKATA T, OTANI T, OHSHIMA S-I and OHNO Y (2000), 'Foods and nutrients in relation to bladder cancer risks: a case-control study in Aichi Prefecture, central Japan', *Nutr Cancer*, 38(1), 13–22.

WALZEM R L, DILLARD C J and GERMAN J B (2002), 'Whey components: millennia of evolution create functionalities for mammalian nutrition: what we know and what we may be overlooking', *Crit Rev Food Sci Nutr*, 42, 353–375.

WANG L, MANSON J E, BURING J E, LEE I M and SESSO H D (2008), 'Dietary intake of dairy products, calcium, and vitamin D and the risk of hypertension in middle-aged and older women', *Hypertens*, 51(4), 1073–1079.

WARENSJÖ E, JANSSON J-H, BERGLUND L, BOMAN K, AHRÉN B, WEINEHALL L, LINDAHL B, HALLMANS G and VESSBY B (2004), 'Estimated intake of milk fat is negatively associated with cardiovascular risk factors and does not increase the risk of a first acute myocardial infarction. A prospective case-control study', *B J Nutr*, 91, 635–642.

WILLETT W C, STAMPFER M J, MANSON J E, COLDITZ G A, SPEIZER F E, ROSNER B A, SAMPSON L A and HENNEKENS C H (1993), 'Intake of trans fatty acids and risk of coronary heart disease among women'. *Lancet*, 341, 581–585.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

WORLD HEALTH ORGANIZATION (2003), *Diet, Nutrition and the Prevention of Chronic Diseases*, WHO Technical Report Series 916.

WU K, WILLETT W C, FUCHS C S, COLDITZ G A and GIOVANNUCCI E L (2002), 'Calcium intake and risk of colon cancer in women and men'. *J Nat Cancer Inst*, 94(6), 437–446.

ZEIGLER E E and FOMON S J (1983), 'Lactose enhances mineral absorption in infancy', *J Pediatr Gastroenterol Nutr*, 2, 288–294.

ZEMEL M B (2003), 'Mechanisms of dairy modulation of adiposity', *J Nutr*, 133, 252S–256S.

ZEMEL M B, SHI H, GREER B, DIRIENZO D and ZEMEL P C (2000), 'Regulation of adiposity by dietary calcium', *FASEB J*, 14, 1132–1138.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

# 2

# The health aspects of milk

**M. de Vrese, M. Pfeuffer, N. Roos, K. Scholz-Ahrens and J. Schrezenmeir, Max Rubner-Institut (MRI) – Federal Research Institute of Nutrition and Food, Germany**

**Abstract:** Cow's milk is not only an important source of calcium, high-value protein, zinc, magnesium and vitamins, but has also scientifically proven health-promoting 'functional' properties. This chapter deals with assured and potential beneficial effects of an adequate milk intake on bone and teeth health, prevention of osteoporosis, the metabolic syndrome, hypertension, and the cardiovascular risk, on overweight and obesity and on gastrointestinal well-being and gut health. Finally, each section of this chapter addresses the question whether the health-promoting value of milk can be or should be enhanced by fortification, modification of the feeding regimen of cows, or addition of components, e.g. probiotic bacteria or prebiotic carbohydrates, to milk products.

**Key words:** health effects of milk, calcium for bone health and osteoporosis prevention, hypertension and overall cardiovascular disease (CVD) risk, overweight and obesity, probiotics, prebiotics and gut health.

IP Address: 129.132.208.100

## 2.1 Introduction

The high nutritional and health-promoting value of milk and fermented milk products has long been known. An Islamic medical textbook recommended (which according to current knowledge may be not quite correct) 'to drink milk, for it wipes away heat from the heart, strengthens the back, increases the brain, augments the intelligence, renews vision and drives away forgetfulness' (cited according to Huth *et al.*, 2006). A Persian version of the Old Testament (*Genesis* 18:8) tells that Abraham owed his longevity to the consumption of sour milk,

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

and in 76 BC the Roman historian Plinius recommended the administration of fermented milk products for treating gastroenteritis (reference cited in Bottazzi, 1983).

Cows' milk is the major source of calcium in many Western-style diets and an important source of some other minerals, of high-value protein and of several vitamins. Milk and milk products (except butter) contribute relevantly to the US food supply of protein (19.4%), calcium (72.1%), phosphorus (32.4%), zinc (16.2%), magnesium (15.8%) and the vitamins $B_2$ (26.1%), $B_6$ (8.7%), $B_{12}$ (21.6%) and A (15.3%) (Gerrior and Bente, 2002).

Furthermore, milk and milk products have a number of health-promoting properties. The beneficial effects on bone and teeth health, hypertension, the metabolic syndrome and cardiovascular disease (CVD) risk, on overweight and obesity and on gastrointestinal well-being and gut health are described in more detail in the following sections. This high nutritional and health-promoting value of milk needs to be maintained during all technological processes to which milk is subjected. If possible, the value should be enhanced by suitable processing technology, by fortification, by modifying the cows' diet or by adding components such as probiotic bacteria or prebiotic carbohydrates to milk products.

## 2.2 Bone and teeth health

### 2.2.1 Calcium and osteoporosis

IP Address: 129.132.208.100

Osteoporosis is an age-related disease characterized by loss of bone mass, mainly trabecular bone, deterioration of the architecture of bone tissue, and consequently increased bone fragility. Estrogen deficiency exacerbates this effect. Accordingly, women after menopause are at higher risk to suffer from bone fractures than men. Osteoporosis is an increasing health problem not only in industrialized countries but also in threshold countries. In Europe the number of osteoporotic fractures and their total direct costs were estimated at 3.79 million cases and €31.7 billion in 2000 (Kanis and Johnell, 2005). In the USA, the economic burden was estimated at more than 2 million fractures and costs of $17 billion in 2005 (Burge *et al.*, 2007). In Germany, the total direct costs attributable to osteoporosis amounted to €5.4 billion in 2003 (Häussler *et al.*, 2007). Some major reasons for this are increasing life expectancy, sedentary lifestyle, improved medical care and changing eating habits, whereas physical activity, especially weight-bearing exercise, sufficient exposure to sunlight to ensure an adequate vitamin D synthesis, and a balanced diet providing enough vitamin D and calcium of high availability, may contribute to prevention of osteoporosis.

Thus, calcium is a lifelong, critical nutrient with respect to bone health. It accounts for 1–2% of adult human body weight. Over 99% of total body Ca is found in the bones and teeth, where, incorporated as hydroxyapatite, it brings about rigidity and also serves as a reservoir for metabolically used calcium. An adequate intake of calcium during childhood and youth increases peak bone

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

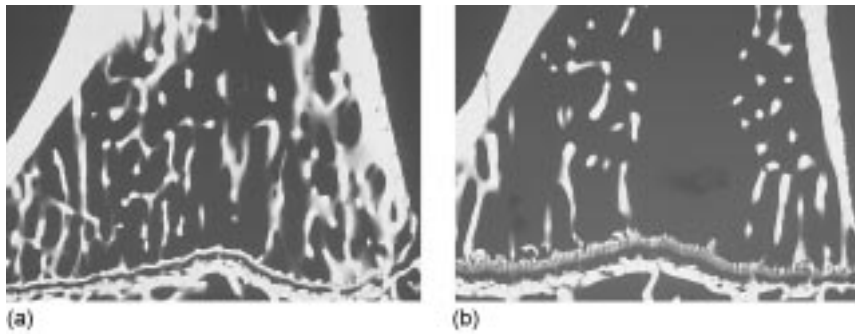



**Fig. 2.1** Trabecular structure of (a) a healthy and (b) an osteoporotic tibia in an osteoporosis rat model (Scholz-Ahrens *et al.*).

mass, whereas in adults and aging people calcium may delay and/or slow down bone resorption, alleviating the risk of reaching a critical or threshold value for bone mineral density, when fracture risk increases exponentially (Weaver, 2008; Dontas and Yiannakopoulos, 2007; Heaney *et al.*, 2000; Fig. 2.1).

### 2.2.2 Calcium requirements and bioavailability

Metabolic balance studies have shown that average absorption rate from whole mixed meals in adults is about 30–35%, and may vary between 25 and 50% depending on age, sex, postmenopausal state, pregnancy, lactation, and calcium and vitamin D status of the body. Calcium homeostasis is regulated by calciotropic hormones, particularly 1,25-dihydroxycholecalciferol, parathyroid hormone and calcitonin. Based on these estimates and considering the adequate safety margins, the recommended dietary allowance (RDA) or adequate intake for calcium varies between 350 and 1300 mg/d (see Table 2.1) to balance the inevitable calcium losses in feces, urine and skin of around 300 mg/d. Actually, men and in particular women ingest calcium at levels which are below these recommendations. The dietary reference intakes (DRI) in the United States are higher than in Europe. At present there is debate on whether current calcium recommendations for adolescents are higher than needed (Atkinson *et al.*, 2008).

### 2.2.3 Regulation of calcium homeostasis

The varying intestinal fractional absorption rates (or availability) of calcium from different foods depend, in addition to the non-nutritive factors mentioned above, on the physico-chemical form of calcium in the food matrix itself. For absorption calcium has to be present in a soluble form. There are two mechanisms by which soluble calcium is absorbed from the gut (Bronner and Pansu, 1999): (1) the passive, nonsaturable transport is based on paracellular diffusion, vitamin D-independent, and has a similar activity throughout the whole intestine; and (2) the active, saturable calcium transport is transcellular, active already at low calcium concentration, displays its main activity in the

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

**Table 2.1** Recommended Ca intakes in different countries

| US (1997) | | UK (1998) | | D-A-CH (2000)[c] | |
|---|---|---|---|---|---|
| Age group (years) | AI/DRI[a] (mg/d) | Age group (years) | RNI[b] (mg/d) | Age group (years) | DRI (mg/d) |
| 1–3 | 500 | 1–3 | 350 | 1–4 | 600 |
| 4–8 | 800 | 4–6 | 450 | 4–7 | 700 |
| | | 7–10 | 550 | 7–10 | 900 |
| 9–18 | 1300 | 11–18 m[d] | 1000 | 10–13 | 1100 |
| | | 11–18 f[d,e] | 800 | 13–19 | 1200 |
| 19–50 | 1000 | 19–50[e] | 700 | 19–50 | 1000 |
| >50 | 1200 | >50 | 700 | >50 | 1000 |

[a] Dietary Reference Intakes 1997.
[b] Reference nutrient intake (Department of Health, 1998).
[c] German, Austrian and Swiss dietary reference intake (Deutsche Gesellschaft für Ernährung, 2000).
[d] m = male; f = female.
[e] An extra amount of calcium (+ 550 mg/d) is recommended for lactating women.

upper small intestine and can be increased by the active form of vitamin D (1,25-dihydroxycholecalciferol), which stimulates the expression of calcium transporters like calbindin D9k.

Thus vitamin D plays an essential role in calcium homeostasis and bone remodeling. Dietary vitamin D (vitamin $D_3$, cholecalciferol) contributes only little (10–20%) to the maintenance of an adequate vitamin D-status, as measured by circulating 25-hydroxycholecalciferol. The majority of 25-hydroxycholecalciferol is of endogenous origin, when 7-dehydrocholesterol is converted to previtamin $D_3$ in the skin upon sunlight (UV) irradiation. Previtamin $D_3$ spontaneously isomerizes to vitamin $D_3$. Vitamin $D_3$, of either dietary or endocrine origin, is hydroxylated in the liver to 25-hydroxycholecalciferol, and the latter in the kidney to its active form, 1,25-dihydroxycholecalciferol, also called vitamin D-hormone (Roux *et al.*, 2008; Dawson-Hughes and Bischoff-Ferrari, 2007). In suckling infants calcium is absorbed by a passive, nonsaturable and vitamin D-independent mechanism. After this period, when calcium density in the diet decreases, the active saturable process gains importance. At low intake levels (<300 mg/d) passive calcium diffusion tends toward calcium secretion into the small intestine. In times of increased calcium requirements, i.e. during pregnancy and breastfeeding, a two-fold increase in calcium absorption rate and a decreased renal calcium excretion maximizes calcium absorption efficiency and minimizes maternal bone loss. Therefore most nutrition councils did not consider increments for pregnancy and lactation in their recent recommendations (see Table 2.1).

In the elderly, in particular in people more than 60 years old, vitamin D status deteriorates as a consequence of less exposure to sunlight, less intake of vitamin D from foods and a decline of renal conversion of 25-hydroxycholecalciferol into the active vitamin D-hormone. Consequently, calcium absorption and utilization decrease and calcium balance becomes negative, which entails a loss

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

of bone mineral and an increased fracture risk. Calcium and vitamin D are thus critical nutrients in this age-group.

### 2.2.4 Bioavailability of calcium from milk and milk products

Milk, yogurt and (ripened) cheese are the most important sources of calcium in Western culture (Table 2.2). These milk products have a high mineral density (mg Ca/kJ), contain calcium in a highly absorbable form, and can be more easily incorporated in sufficient amounts into a diversified diet than other inexpensive calcium sources like kale, parsley or sesame seed. In Western diets about 60% of calcium is provided by milk and milk products (Huth *et al.*, 2006).

Milk contains 1320 mg calcium per liter and is the food with the highest nutrient density for calcium. One half-liter of milk, yogurt or kefir provides approximately 65% of the recommended daily allowance of calcium of 1000 mg for adults. This calcium is highly absorbable and bioavailable (see Table 2.3).

It is widely assumed that the bioavailability of calcium in milk and dairy products is markedly higher compared with other foods or inorganic calcium salts. This has been explained by the physico-chemical form of calcium in milk, which is micellar and colloidal, and the favorable Ca:P ratio; furthermore the occurrence of calcium absorption-enhancers like caseinophosphopeptides, lactose, citrate, and vitamin D, and the absence of antinutritive factors like phytate and oxalate contribute to its high bioavailabilty. As a matter of fact

**Table 2.2** Calcium concentration in foods

| Product | Serving size (g) | mg Ca per 100 g product | mg Ca per serving |
|---|---|---|---|
| Fresh milk (3.5%) | 200 | 120 | 240 |
| Yogurt (3.5%) | 150 | 120 | 180 |
| Skim milk yogurt (0.2%) | 200 | 140 | 210 |
| Cream/Crème fraiche (30%) | 30 | 80 | 26 |
| Fruit whey drink | 200 | 100 | 200 |
| Buttermilk | 200 | 110 | 220 |
| Cheddar (50%) | 30 | 900 | 270 |
| Gouda/Edam/Tilsiter (45%) | 30 | ~800 | 240 |
| Camembert (50%) | 40 | 350 | 140 |
| Feta (50%) | 40 | 450 | 180 |
| Curd cheese (20%) | 50 | 120 | 60 |
| Mozzarella | 50 | 450 | 225 |
| Curly kale (raw) | 200 | 212 | 424 |
| Fennel | 200 | 109 | 218 |
| Broccoli/leek | 200 | 87 | 174 |
| Parsley | 15 | 246 | 37 |
| Oranges/berries | 200/150 | 33/44 | 65 |
| Bread (pumpernickel) | 50 (1 slice) | 110 | 55 |
| Whole grain bread | 50 (1 slice) | 84 | 42 |
| Hazelnuts/almonds | 50 | 240 | 120 |

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded From Vet-eBooks.com

**Table 2.3** Calcium bioavailability from food and calcium salts

| | Ca/100 g (mg) | Fractional absorption (%) | Reference |
|---|---|---|---|
| Milk | 125 | 32 | Weaver *et al.* (1999) |
| Cheddar | 720 | 32 | Weaver *et al.* (1999) |
| Yogurt | 125 | 40 | Weaver *et al.* (1999) |
| Beans (red) | 23 | 24 | Weaver *et al.* (1999) |
| Broccoli | 50 | 61 | Weaver *et al.* (1999) |
| Curly kale | 212 | 40 | Weaver *et al.* (1999) |
| Spinach | 135 | 5 | Weaver *et al.* (1999) |
| Mineral water | | 32 | Couzy *et al.* (1995) |
| Calcium lactogluconate | 11 | 37 | Werner *et al.* (1999) |
| Calcium citrate malate | 30 | 36 | Heaney *et al.* (1990) |
| Tricalcium phosphate | 38 | 25 | Heaney *et al.* (1990) |
| Calcium carbonate | 40 | 30 | Heaney *et al.* (1990) |

fractional absorption from milk products is much higher than from most foods of plant origin. Moreover, the daily dairy servings required to meet a defined amount of calcium are much smaller and thus their incorporation into the daily diet is easier to achieve than solely from staple foods like beans, kale, spinach, potatoes or bread (Weaver *et al.*, 1999).

IP Address: 129.132.208.100

With respect to availability, the majority of studies do not show physiologically relevant differences between calcium from milk and milk products or from highly soluble, simple or complex calcium salts like calcium chloride, tricalcium citrate, calcium-citrate-malate or calcium lactogluconate. Even calcium salts that are poorly water-soluble like calcium carbonate, calcium oxide or calcium monophosphates are accepted low-cost sources of calcium, except in cases of achlorhydria, and provided that the single dose does not exceed 500 mg (Nickel *et al.*, 1996; Straub, 2007).

Explanations for this are that other factors negate or overcompensate for the effect of solubility, that the poorer absorption of calcium from carbonate may be leveled out in acidic gastric medium, and that calcium phosphate and the calcium–casein complex in milk are present as colloidal aggregates, which are reported to be similarly or even better absorbed than ionic calcium. To date, the assumption that fermented milk products exhibit better calcium bioavailability than milk because of their lactic acid content has not been confirmed.

The Ca : P ratio of the diet and the content of calcium and phosphorus in absolute terms both affect calcium and bone metabolism (Masuyama *et al.*, 2003; Koshihara *et al.*, 2004). An increase of dietary calcium due to strategies for osteoporosis prevention should consider adequate intake of phosphorus. Recent reports underline that 10–15% of older women have intakes of less than 70% of the recommended daily allowance of phosphorus (Heaney, 2004). This

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

point should receive more attention because there is an increasing demand and a large market for calcium supplements, and these are mainly carbonate or citrate salts (Heaney, 2004). In contrast to calcium from milk, the use of isolated mineral supplements may exceed the dietary Ca:P ratio and, moreover, may imbalance other minerals and trace elements and thus impair their absorption. Milk and milk products contain phosphorus, mainly in the form of phosphate and phosphoric acid esters. The Ca : P ratio in milk is 1.3 w/w and is thus very close to the national guidelines of Germany, Austria and Switzerland of 1.4 w/w (Referenzwerte für die Nährstoffzufuhr, 2000), which is optimal to build up hydroxyapatite. Hydroxyapatite is essential for mineralization, rigidity and structure of bone, and its Ca : P ratio is about 1.7 w/w. Furthermore, phosphorus may counteract protein-induced hypercalciuria.

However, when considering bone health, excess phosphorus intake should be avoided, because the resulting high serum phosphate concentration causes a (transient) drop in serum-ionized calcium and increased PTH secretion, and thereby potentially causes bone resorption. Adolescents are at risk of not achieving an optimum peak bone mass when they change dietary habits, i.e. when they exchange milk for soft drinks, because the latter contain high amounts of phosphorus but no calcium. With respect to bone health, some milk products should be preferred compared to those that contain higher amounts of phosphorus or sodium, for technological reasons.

Milk contains other minerals and vitamins relevant for bone health. Half a liter of milk supplies a large percentage of the recommended daily intakes of many minerals and vitamins, such as magnesium (16%), potassium (15%), zinc (20%), and vitamin D (9%) (Dietary Reference Intakes, 1997, 2001, 2005).

IP Address: 129.132.208.100

Intestinal absorption of calcium may be affected by concomitant ingestion of digestible carbohydrates. A stimulating effect of lactose is well documented in children, whereas in healthy adults there was little or no effect. Whereas increased calcium absorption due to lactose ingestion has been described in lactose absorbers, impaired calcium absorption has been observed in lactose maldigesters (Obermayer-Pietsch *et al.*, 2007).

A high protein intake increases renal calcium excretion (Kerstetter and Allen, 1994) due to a significant increase in the glomerular filtration rate and a decrease in renal tubular reabsorption rather than to increased calcium absorption (Benable and Martinez-Maldonado, 1991). This calciuric effect depends mainly on acidogenic components of dietary protein, especially on the sulfur-containing amino acids, which are metabolized to sulfate. Milk protein, however, contains less sulfur-containing amino acids than typical animal proteins and is considered to be 'neutral' rather than acidogenic. Any remaining calciuric protein effect may be compensated by the hypocalciuric effect of phosphate in milk.

Casein, the major milk protein, is hydrolyzed in the digestive tract, where encrypted phosphopeptides ('caseinophosphopeptides', CPP) are liberated. CPP have been found to increase the solubility of calcium in the small intestine (Matsui *et al.*, 1997), a pre-requisite for its absorption. Depending on the experi-

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

mental conditions, this effect was demonstrated in some but not in all studies (Scholz-Ahrens and Schrezenmeir, 2000; Bouhallab and Bouglé, 2004; Möller *et al.*, 2008).

Other encrypted peptides or protein fractions that improve bone health have been described. It was shown that whey protein, especially its basic fraction (the so-called 'milk basic protein', MBP) or some of its components, promoted bone formation and suppressed bone resorption (Aoe *et al.*, 2001, 2005) at intakes of less than 50 $\mu$g MBP per day. The underlying mechanisms are not yet fully understood. Moreover, it is not clear whether the quantity of MBP that is ingested with normal intakes of milk contributes significantly to a health effect on bone. The presence of encrypted MBP within milk could, however, be a partial explanation for the greater beneficial effect of milk and milk products on bone health in some studies, as compared to calcium supplements.

### 2.2.5 Calcium and milk products for bone health and prevention of osteoporosis

To fulfill their many functions such as adaptation to varying skeletal strain patterns, repair of bone microdamage or maintenance of calcium homeostasis, bones are subject to lifelong 'remodeling', a coupling of bone resorption and formation by specific cells (osteoclasts and osteoblasts, respectively) with little change in shape. Calcium intake or dairy consumption is positively associated with bone mineral density, a predictor of bone fracture risk (see Fig. 2.2). There is considerable evidence from intervention studies and less stringent evidence from observational studies showing that a lifelong, high calcium intake together with sufficient physical activity and strain on bones (1) increases gain of bone mineral and peak bone mass in adolescents and young adults, (2) preserves bone density in adults, and (3) attenuates bone loss in the elderly, particularly in women after the menopause (Heaney *et al.*, 2000; Heaney, 2007; Adolphi *et al.*, 2009).

IP Address: 129.132.208.100

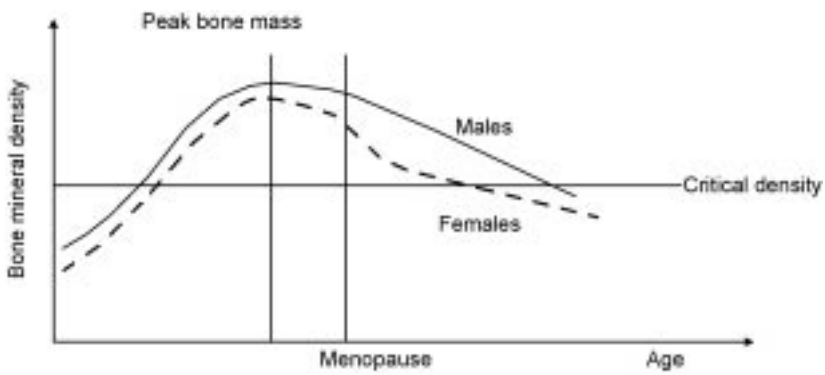



**Fig. 2.2** Course of bone mineral density (BMD) throughout life. Critical bone mineral density is achieved earlier in females than in males because of an accelerated loss of bone mineral with the onset of menopause due to the decline in estrogen production.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

Some intervention trials, however, failed to show relevant effects of high calcium or dairy intake on bone health (Lanou *et al.*, 2005), and some meta-analyses showed only a weak association between calcium or dairy intake and bone mass, fracture rates or the incidence of osteoporosis (Tang *et al.*, 2007; Winzenberg *et al.*, 2006). The reason for this is the slow development of osteoporosis over years and the large number of risk factors and confounders that are involved but are difficult or impossible to control, like the calcium and/or vitamin D status of the study participants, their habitual diet with great variation of the amount and source of calcium, and other foods that interfere with calcium absorption. The study period may have been too short, and investigation of the 'wrong' bone may have played a role: strained bones (tibia, fibula) profit predominantly from physical activity, while non-strained bones (humerus, ulna, radius) profit more from an increased calcium intake, and for the femur only physical activity plus calcium shows a significant positive effect (Wosje and Specker, 2000).

### 2.2.6 Milk products and teeth health

Tooth enamel is composed largely of hydroxyapatite ($Ca_5(PO_4)_3(OH)$) crystals and is therefore susceptible to acids and calcium-complexing agents. It is always attacked if fermentable sugars are metabolized to organic acids by certain plaque bacteria (especially *Streptococcus mutans*), which decreases the pH of the tooth surface below 5.6.

Milk is only weakly cariogenic or probably not cariogenic at all (Bowen and Lawrence, 2005). Only half of the lactose molecule (namely glucose) is quickly fermented. Therefore, lactose decreases the pH less than an equivalent amount of sucrose or glucose. The high calcium and phosphate content of milk and the buffering capacity of milk proteins inhibit enamel dissolution and demineralization of the teeth and promote or increase remineralization. Ripened cheese and particularly hard and semi-hard cheese is even considered to be the most anticariogenic food and protective against coronal as well as root caries (Kashket and DePaola, 2002). This may be due to one or more of the following mechanisms: stimulation of saliva by cheese chewing, buffering of cariogenic acids, inhibition of enamel demineralization and enhancement of remineralization through the high concentrations of casein, calcium and phosphate, which are higher in cheese than in milk, and the bacteriostatic or even bacteriocidal properties of milk proteins or calcium.

IP Address: 129.132.208.100

### 2.2.7 Improving bone- and teeth-protective properties of milk and dairy products

Many individuals do not consume the amount of calcium recommended for prevention of osteoporosis. Milk and calcium-rich dairy products can contribute significantly to the supply of adequate calcium intake of the population, either by increased consumption of dairy products or by offering new functional milk products that are fortified with calcium and/or absorption enhancers.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

In populations with a high incidence of lactose maldigestion and intolerance, dairy consumption could be raised by means of lactose-reduced or lactose-free dairy products, particularly cheese and lactose-hydrolyzed and fermented milk products containing active microbial $\beta$-galactosidase (see page 53). Whether removal of lactose has a measurable negative impact on intestinal calcium absorption has not been demonstrated to date.

Several measures are conceivable to improve calcium absorption and bioavailability. These include (1) fortification of milk and dairy products with CPP or other relevant peptides; (2) addition of prebiotic fructo-oligosaccharides, i.e. undigestible carbohydrates, which, upon ingestion, will be fermented by the intestinal microbiota, leading to acidification of intestinal contents and improved calcium solubility and absorption (Scholz-Ahrens *et al.*, 2001, 2002, 2007; Scholz-Ahrens and Schrezenmeir, 2007); and (3) vitamin D fortification of dairy products. The latter is effective and is used frequently in many Western countries to improve calcium bioavailability in addition to the aimed improvement of vitamin D status in general. In other countries (e.g. Germany) vitamin D fortification is forbidden because of the risk of exceeding the tolerable upper intake levels (UIL) of 50 $\mu$g vitamin D in Europe and the USA (Przyrembel, 2005; Dietary Reference Intakes, 1997).

### 2.2.8 Conclusions: calcium-fortified milk products – redundant or helpful

In Germany and some other countries, calcium fortification of milk products is rejected by health authorities. It is argued that milk already contains a high concentration of available calcium, and milk-avoiders, who are most exposed to the risk of low calcium intake, would not profit from these products. It is also thought that calcium fortification of milk products would increase the risk of exceeding tolerable UILs for calcium, i.e. 2500 mg/day in Europe and the USA (Przyrembel, 2005; Dietary Reference Intakes, 1997). On the other hand, fortification is promoted with the argument that many persons would find it difficult to reach the newly recommended intake of up to 1300 mg/d without calcium supplements or fortified foods. Furthermore, the risks of exceeding the UIL for calcium are lowest when dairy foods are fortified, as they are known to be good sources of calcium even without fortification, instead of arbitrarily fortifying a random selection of foods. This question needs further discussion.

IP Address: 129.132.208.100

## 2.3 Hypertension and overall cardiovascular disease (CVD) risk

### 2.3.1 General aspects

It is now recognized that a number of factors contribute to an enhanced risk of cardiovascular disease (CVD). A cluster of metabolic disorders that enhance CVD risk is termed the metabolic syndrome (MS). These include obesity, hypertension, hyperlipidemia (increased low-density-lipoprotein (LDL) and/or decreased high-density-lipoprotein (HDL) cholesterol and increased triglyceride

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

**Table 2.4** Defining the metabolic syndrome

| Parameter | Threshold | |
|---|---|---|
| | ATP III[a] Insulin insensitivity (high glucose) plus two or more of the following symptoms | IDF (Europeans)[b] Three or more of the following symptoms |
| Waist circumference (cm) | ≥102 (m), ≥88 (f) | ≥94 (m), ≥80 (f) |
| Blood pressure (mm Hg) | ≥135/85 | ≥130/85 |
| Glucose (mg/dl) | ≥100 | ≥100 |
| Triglyceride (mg/dl) | ≥150 | ≥150 |
| HDL-cholesterol (mg/dl) | ≤42 (m), ≤50 (f) | ≤40 (m), ≤50 (f) |

[a] Adult Treatment Panel III (National Institutes of Health 2001); m = males, f = females, mg/dl = milligrams per deciliter.
[b] www.idf.org

levels), insulin insensitivity or diabetes, and atherosclerosis. Obesity is at the forefront, and insulin insensitivity is the core phenomenon. According to the Adult Treatment Panel of the American Heart Association (ATP III) (Grundy *et al.*, 2005), a metabolic syndrome is established if insulin resistance comes together with two or more of the other disorders. The limits defined by the International Diabetes Federation are even stricter (Table 2.4). Beyond that, there are other disorders like high homocysteine or uric acid levels that may play a role in MS.

CVD refers to a class of diseases that involve the heart or blood vessels related to atherosclerosis. According to the 'response to injury' theory (Ross, 1993), pathogenesis of atherosclerosis starts with endothelial dysfunction in blood vessels induced by the aforementioned factors, as well as smoking and bacterial toxins. These factors excite a chronic inflammation as a defense reaction. Over years or even decades, advanced lesions (atherosclerotic plaques) develop and narrow the lumen of the blood vessels. Sudden plaque rupture causes acute myocardial infarction and stroke.

Epidemiological data give little indication that milk and dairy consumption is linked with increasing risk of CVD. A number of studies even indicate that the risk may be reduced with increasing dairy intake. A meta-analysis of 10 studies published before 2002 found that all but one study suggested a lower risk in subjects with the highest intake of milk compared to subjects with the lowest intake (Elwood *et al.*, 2004). Relative risk compared to subjects with the lowest consumption was 0.87 for heart disease and 0.83 for stroke. These results relate to the consumption of mainly full-fat milk, as at the time when these studies were carried out more full-fat than low-fat milk was on the market. More recent cohort studies confirmed these findings. Case-control studies observed also a significant inverse association between yogurt consumption and coronary heart disease (Tavani *et al.*, 2002) and between milk intake and the risk of a first myocardial infarction (Warensjö *et al.*, 2004).

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

### 2.3.2 Milk and hypertension

Hypertension is a major risk factor for CVD. Individuals with high blood pressure are three to four times more likely to develop coronary heart disease. Hypertension is the strongest risk factor for stroke. Even persons with high-normal blood pressure have a significantly increased risk of CVD compared to persons with ideal levels. Weight loss, reduction in sodium intake, increased physical activity, and limited alcohol intake are established recommendations that reduce blood pressure.

A number of prospective studies suggest that dairy intake may favor low blood pressure. In the CARDIA study population, the risk for 10-year cumulative incidence of hypertension in the highest dairy intake category ($\geq$35 times per week) was less than 50% of that in the lowest category (<10 times per week). The inverse relation was more obvious in obese persons (Pereira *et al.*, 2002). A follow-up study over 15 years, including a total of 4300 subjects, found an inverse trend for dairy intake ($p = 0.06$). Food subgroup analysis showed a significant inverse trend for milk and dairy desserts, but not for cheese (Steffen *et al.*, 2005). The publication gives no information as to what types of cheese the participants consumed, but as this is a study from the USA, it may be assumed that more fresh and less ripened varieties were consumed. Only the latter may contain relevant amounts of hypotensive peptides (see below). For more detailed coverage see reviews by Jauhiainen and Korpela (2007) and Pfeuffer and Schrezenmeir (2007). A DASH intervention trial (Dietary Intervention to Stop Hypertension) showed that a diet rich in fruits, vegetables and low-fat dairy foods reduces blood pressure significantly more than the respective diet without dairy foods in individuals with both optimal and elevated blood pressure (Appel *et al.*, 1997). Inclusion of dairy products nearly doubled the effect of fruits and vegetables. The benefit of following the DASH dietary pattern was shown to apply throughout the range of sodium intakes (Sacks *et al.*, 2001). In another study a supplement of skim milk decreased blood pressure in subjects with both optimal and elevated blood pressure (Buonopane *et al.*, 1992).

Several components of milk and dairy products may contribute to the blood pressure-reducing effect. The role of antihypertensive milk peptides is without question. Peptides containing up to 10 amino acids may be released from milk proteins through the proteolytic activity of lactic acid bacteria, and are thus found in fermented dairy products, or they may be released during the digestive process in the gut. The hypotensive potential of the peptides requires that they reach target sites without being degraded, through, for example, intestinal or plasma peptidases. Probably the most important underlying mechanism of the antihypertensive effects of milk peptides is inhibition of angiotensin-converting enzyme (ACE) (FitzGerald and Meisel, 2000). Milk peptides may also exert an effect via endothelin release-inhibitory activity. A blood pressure-reducing effect in hypertensive rats was obtained by feeding milk fermented with *Lactobacillus helveticus* CP 790 or *Saccharomyces cerevisiae*, or by feeding two purified tripeptides, Val-Pro-Pro and Ile-Pro-Pro. Fermented milk products in amounts of 95–150 ml/d reduced blood pressure in humans. A powdered, *Lb.*

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

*helveticus* CM4-fermented milk, providing 13 mg Val-Pro-Pro and Ile-Pro-Pro per day, reduced systolic blood pressure in subjects with high-normal and mildly elevated blood pressure (Aihara *et al.*, 2005).

Furthermore, calcium in milk may have a blood pressure-reducing effect. Numerous animal and human epidemiologic and intervention studies observed an inverse relationship between dietary calcium and blood pressure. Besides calcium, intake of magnesium and potassium is inversely correlated with blood pressure (Massey, 2001). It was assumed that calcium may suppress circulating vasoactive, calcium-regulating hormones. Lactose, citrate, milk proteins and peptides may improve bioavailability of calcium from milk.

Conjugated linoleic acid (CLA) supplementation reduced blood pressure in spontaneously hypertensive rats (Inoue *et al.*, 2004) and prevented onset of hypertension in fatty, diabetic rats (Nagao *et al.*, 2003). The effects were attributed to the ability of CLA to regulate the production of adipocytokines, such as adiponectin, leptin and angiotensin. There was no effect of CLA on blood pressure in most human studies. Only in pregnant women who consumed 600 mg calcium plus 450 mg CLA daily was pregnancy-induced hypertension significantly reduced (Herrera *et al.*, 2006).

### 2.3.3 Other factors in milk and milk products with a positive impact on the CVD risk

*Obesity*

The relationship between milk and obesity is addressed in Section 2.4.

IP Address: 129.132.208.100

*Lipids*

Unlike the situation some years ago, low HDL cholesterol and high triglyceride levels rather than high total plasma cholesterol levels are now the main biomarkers used when assessing CVD risk factors (Grundy *et al.*, 2005; Table 2.4). Milk is often considered a potential promoter of atherosclerosis and CVD because it is a source of cholesterol and saturated fatty acids. Butter consumption increases not only total cholesterol, but also HDL cholesterol, such that the ratio of total to HDL cholesterol (or of LDL to HDL cholesterol) is usually not impaired, i.e. not increased. Furthermore, early intervention studies showed that skim milk or yogurt consumption might actually decrease plasma cholesterol levels, while whole milk had neither a hypo- nor a hypercholesterolemic effect. Recent observational studies confirm that higher consumption of milk products is associated with lower cholesterol levels (Pereira *et al.*, 2002; Samuelson *et al.*, 2001). The DASH dietary regimen decreased total and LDL cholesterol beyond the control diet containing no dairy foods (Obarzanek *et al.*, 2001).

It is not clear yet what component(s) play the part of a hypocholesterolemic 'milk factor'. Calcium, milk peptides and phospholipids are prime candidates. Calcium might have a hypolipidemic effect by decreasing intestinal absorption of cholesterol, of bile acids, and of fat (Shahkhalili *et al.*, 2001). Arguments for the role of milk proteins or peptides are that, at equal fat content, cheese

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

consumption decreased total and LDL cholesterol as compared to butter (Biong *et al.*, 2004), and that fermented milk products decreased cholesterol levels more than non-fermented products (Agerhol-Larsen *et al.*, 2000). Certain strains of (probiotic) bacteria seem to be more efficient than others (Xiao *et al.*, 2003). Phosphatidylinositol (contained in soy as well as in milk phospholipids) increased levels of HDL cholesterol and apolipoprotein A1 (major apolipoprotein of HDL) and decreased plasma triglycerides. The effect was more pronounced when the preparation was administered together with a light meal (Burgess *et al.*, 2005). Unlike plant phospholipids, milk is also a rich source of sphingomyelin. Sphingomyelin is an inhibitor of pancreatic lipase–colipase and may also decrease micellar solubilization of cholesterol and thus its absorption efficiency. Probably due to its higher degree of saturation, milk sphingomyelin was more effective than egg sphingomyelin in inhibiting absorption of cholesterol and fat in rats (Noh and Koo, 2004). It is not clear if this mechanism is relevant in humans. During processing, phospholipids remain largely with the protein rather than with the fat fraction. Therefore, both full-fat and low-fat milk possess hypolipidemic potential. Butter is relatively depleted of phospholipids.

Dairy fat can be enriched with mono- and polyunsaturated fatty acids at the expense of saturated fatty acids by modified cow feeding regimens. Consumption of modified as compared to a regular milk fat reduced total and LDL cholesterol by 7.9% and 9.5%, respectively, in healthy, normo-cholesterolemic and normal-weight men (Poppitt *et al.*, 2002). The effect was more pronounced than would be predicted from the change in the fatty acid pattern. More studies are needed to explore this effect further. That small changes in the milk fatty acid pattern may have measurable effects on lipid levels is supported by studies that examined the effect of small amounts of long-chain $\omega$3 fatty acids (eicosapentaenoic acid, 20:5$\omega$3, and docosahexaenoic acid, 22:6$\omega$3) added to skimmed or semi-skimmed milk. Consumption of 0.5 l/d of such milks, providing between 200 and 300 mg/d $\omega$3 fatty acids and administered for several months, reduced lipid levels (Carrero *et al.*, 2007; Benito *et al.*, 2006).

IP Address: 129.132.208.100

Plasma triglyceride levels increase after a fatty meal and return to baseline 6–12 hours later. A high post-meal triglyceride response is associated with increased CVD risk (Cohn, 1998). It is higher in obese and insulin-resistant than in lean and non insulin-resistant subjects, due to an accumulation of triglyceride-rich atherogenic remnant lipoproteins. This post-meal triglyceride response is usually more pronounced with saturated rather than polyunsaturated fatty acids. But frequently the response to milk fat in a mixed meal was comparable to, or less than, that produced by an oil rich in polyunsaturated fatty acids. This attenuated response is most probably due to short-chain and medium-chain fatty acids in dairy fat (see Marten *et al.* 2006 for more details).

*Insulin sensitivity*

As mentioned before, insulin insensitivity or insulin resistance is the core phenomenon of the metabolic syndrome. In the Health Professionals Follow-up Study, which followed 41,254 healthy male participants for 12 years, each

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

serving-per-day increase in total dairy intake was associated with a lower risk for type 2 diabetes (relative risk 0.91). The corresponding relative risk was 0.88 for low-fat dairy intake (Choi *et al.*, 2005). In an observational study, fasting glucose and post-meal glucose increase also was lower with higher fat intake from milk and cream (Smedman *et al.*, 1999). A DASH dietary pattern in humans improved insulin sensitivity beyond the effects of the control diet not including low-fat dairy foods (Ard *et al.*, 2004).

Milk proteins, especially whey protein, probably play a role in these effects. When insulin-resistant rats were fed whey or meat protein diets, whey protein reduced plasma insulin and increased insulin sensitivity (Belobrajdic *et al.*, 2004). When rats were fed a high-fructose diet together with probiotic yogurt containing *Lb. acidophilus* and *Lb. casei* (dahi), levels of glucose, insulin and glycated hemoglobin (HbA1c) were reduced as compared to fructose-fed control rats. Indicators of oxidative stress and lipid levels were also significantly improved, but weight increase was identical to that in the fructose-fed control group (Yadav *et al.*, 2007). In rats given a sucrose-rich meal, substitution of $\alpha$-lactalbumin for whole milk protein attenuated the post-meal glucose increase and improved insulin sensitivity. Non-milk proteins were not tested in this study (Blouet *et al.*, 2007).

Dairy fat is unusual in that it contains a relatively large amount of short-chain (4:0) and medium-chain (6:0-12:0) fatty acids, on average 3.6% and 9.7%, respectively, by weight. Medium-chain fatty acids may affect insulin sensitivity directly, through regulation of glucose metabolism, and indirectly, through regulation of adipose tissue metabolism. Mechanistic studies indicate that longer-chain, saturated fatty acids impair glucose metabolism, but medium-chain fatty acids can actually improve it (for more details see Marten *et al.*, 2006). Evidence that medium-chain fatty acids affect adipocyte metabolism beneficially will be outlined in the section on obesity.

IP Address: 129.132.208.100

*Inflammation*

Insulin resistance and diabetes, obesity, hypertension and also dyslipidemia (especially low HDL-cholesterol levels, together with low apolipoprotein A1 levels) are associated with a proinflammatory state of the body. Elevated homocysteine levels (tHcy) are an indicator of such a proinflammatory state (Gori *et al.*, 2005). Hypertensive subjects have higher levels of tHcy as well as lower levels of the endogenous antioxidant, glutathione (GSH) (Rodrigo *et al.*, 2007). Conversely, homocysteine-induced endothelial dysfunction can be reversed by increasing the concentration of GSH. The tripeptide GSH (L-glutamyl-L-cysteinyl-glycine) is the most important intracellular antioxidant of the body.

Observational studies indicate that higher consumption of dairy foods is associated with lower homocysteine levels (Lutsey *et al.*, 2006; more references in Pfeuffer and Schrezenmeir, 2007). In a DASH intervention study, including milk products in the diet reduced serum homocysteine levels beyond those of a diet rich in fruits and vegetables only (Appel *et al.*, 2000).

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

Among milk components, proteins and phospholipids may have a beneficial role. Whey proteins, especially $\alpha$-lactalbumin, are a rich source of cysteine. Whey protein (45 g/d) increased plasma GSH levels in humans (Micke *et al.*, 2002) and abolished fructose-induced impaired GSH status in rats (Yadav *et al.*, 2007). Phosphatidylcholine also improved GSH levels in children with cystic fibrosis (Innis *et al.*, 2007) and decreased homocysteine levels in healthy subjects (Olthof *et al.*, 2005). Some effects of phospholipids are probably independent of the source, i.e. independent of phospholipid fatty acid pattern. But in some respects phospholipids rich in saturated fatty acids (present in milk) may be more effective than more unsaturated phospholipids (predominant in soybean oil). In a mouse model, dimyristoyl-phosphatidylcholine (found in milk phospholipids) increased apolipoprotein A1 levels and HDL-cholesterol and prevented atherosclerotic lesions in mice more than equivalent amounts of egg or soy phospholipids (Navab *et al.*, 2003).

Medium-chain fatty acids may attenuate or protect from inflammation (see Marten *et al.*, 2006). The clinical relevance of these findings remains to be established. An interesting aspect is that medium-chain fatty acids have a beneficial effect on serum and tissue levels of long-chain $\omega$3 fatty acids, which have anti-inflammatory properties. Furthermore, a number of studies indicate that dairy foods may improve the bioavailability of folate. Higher dairy consumption was associated with higher folate levels in observational studies. The DASH dietary regimen not only decreased serum homocysteine levels but also increased folate levels (Appel *et al.*, 2000). In other studies, low-dose $\omega$3 fatty acids (up to 350 mg/d) combined with low-dose folic acid (150 $\mu$g/d) supplements in 500 ml/d milk decreased homocysteine and increased folate levels (Carrero *et al.*, 2007; Benito *et al.*, 2006) and improved plasma levels of long-chain $\omega$3 fatty acids markedly (Carrero *et al.*, 2007). Cell culture and animal studies suggest that CLA has anti-inflammatory properties (Bhattacharya *et al.*, 2006). In human studies, however, no such effects were observed.

IP Address: 129.132.208.100

Obesity and the metabolic syndrome change the serum phospholipid fatty acid pattern toward higher levels of saturated and lower levels of ω3 fatty acids (Klein-Platat, 2005). Although milk fatty acids may increase serum cholesterol, they apparently have no negative effect on serum phospholipid fatty acid pattern in humans. This was shown for butter as compared to soybean oil (Lichtenstein *et al.*, 2003). Higher consumption of dairy fat (indicated by the concentration of pentadecanoic acid) was associated with higher levels of long-chain $\omega$3 fatty acids in serum phospholipids and cholesterol esters in an observational study (Smedman *et al.*, 1999). Some reports suggest that conjugated linoleic acid (CLA) improves metabolism of $\omega$3 fatty acids (Attar-Bashi *et al.*, 2007).

### 2.3.4 Conclusions: milk products protecting from hypertension and CVD risk

Milk is a whole food with many beneficial components and should therefore be an essential component of a varied healthy diet. Differently processed products

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

have different effects. There are interactions between milk products and other foods. Therefore not much can or needs to be done to improve milk products. Fermented products may be particularly effective in reducing blood pressure. Low-fat products carry almost all the benefits, as phospholipids remain largely with the protein fraction. When it comes to milk fat, it may be a promising approach to induce a shift in the fatty acid pattern by modifying feeding regimens of the cows, e.g. by including rapeseed cake, in order to reduce the content of saturated fatty acids somewhat and to increase the concentration of vaccenic, oleic and conjugated linoleic acid.

## 2.4 Protection from obesity

### 2.4.1 Milk and obesity

The potential association between dairy product consumption and body weight regulation has been the subject of several recent publications. Data from cross-sectional epidemiological studies (Zemel *et al.*, 2000; Carruth and Skinner, 2001; Pereira *et al.*, 2002), rather than prospective studies, and randomized controlled intervention trials with sufficiently large subject numbers showed an inverse association between a high intake of milk and milk products and body weight or body fat accumulation. In a small study, body mass and waist circumference were inversely related to milk fat intake (Smedman *et al.*, 1999).

One may argue that high consumption of milk and milk products might be a marker of a healthier lifestyle, and that methodological weaknesses like insufficiently precise recording of the true intake of individual dairy products and of the nutritional differences between them might have biased the study results. It should also be mentioned that the energy density of many dairy products is actually very low (Table 2.5). But, on the other hand, many of the available data support – though not unequivocally – the hypothesis that one or more milk constituents, e.g. calcium, CLA, medium-chain fatty acids or appetite-regulatory whey proteins and bioactive peptides released from the proteins, are causally related to lower fat mass deposition by decreased absorption and/or higher utilization of fat or energy, respectively.

IP Address: 129.132.208.100

### 2.4.2 Calcium

*Evidence for an inverse relation between calcium intake and obesity*

Observations showing an inverse relationship between calcium intake and obesity have been published since the end of the 1980s, but this hypothesis became more popular in the scientific community following the seminal paper by Zemel and co-workers (Zemel *et al.*, 2000). This publication was based mainly on investigations in obese and insulin-resistant mouse mutants ('agouti mouse') and led to an intensive re-examination and extended interpretation of data from several epidemiological studies.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

**Table 2.5** Energy density of selected foods

| Product | Fat (g/100 g) | Energy density (kJ/100 g) | Product | Fat (g/100 g) | Energy density (kJ/100 g) |
|---|---|---|---|---|---|
| Milk (3.5%) | 3.5 | 278 | Beef fillet | 4.0 | 506 |
| Milk (1.5%) | 1.5 | 206 | Pork fillet | 2.0 | 445 |
| Milk (0.2%) | 0.2 | 153 | Chicken breast | 6.0 | 605 |
| Yogurt (3.5%) | 3.5 | 308 | Salami | 50 | 2191 |
| Yogurt (1.5%) | 1.5 | 212 | Wiener | 22 | 1150 |
| Yogurt (0.2%) | 0.2 | 176 | Herring, cooked | 17 | 972 |
| Fruit yogurt (3.5%) | 3.5 | 473 | Coalfish, cooked | 1.0 | 377 |
| Fruit yogurt (0.2%) | 0.2 | 363 | Chicken egg, whole | 11 | 636 |
| Whipped cream (30%) | 30 | 1296 | Pasta prepared with eggs | 2.7 | 1505 |
| Milk ice-cream | 2.4 | 356 | Potatoes, cooked | 0.1 | 71 |
| Kefir | 3.5 | 290 | Rice, not hulled | 2.2 | 1463 |
| Curd cheese (40%) | 11 | 692 | Salad vegetables | 0.2 | 12 |
| Curd cheese (20%) | 5 | 470 | Broccoli | 0.2 | 27 |
| Curd cheese (2%) | 0.5 | 319 | Leek | 0.4 | 26 |
| Brie (60%) | 33 | 1622 | Legumes | 1.4 | 280 |
| Chester (50%) | 32 | 1765 | Wholemeal bread | 1.0 | 841 |
| Emmental (45%) | 30 | 1741 | with sunflower seeds | 3.0 | 901 |
| Mozzarella (45%) | 20 | 1162 | White bread | 1.3 | 240 |
| Gouda (45%) | 30 | 1616 | for toasting | 3.4 | 1080 |
| Camembert (40%) | 20 | 1223 | Apple, fresh | 0.4 | 221 |
| Parmesan (30%) | 22 | 1601 | Hazelnuts | 62 | 2849 |
| Cottage cheese (10%) | 2.9 | 376 | Milk chocolate | 31 | 2241 |

Source: German nutrient database II.3, 2007.

IP Address: 129.132.208.100

Data from US NHANES III (National Health and Nutrition Examination Survey; Zemel *et al.*, 2000), the CSFII study (Continuing Survey of Food Intake by Individuals; Albertson *et al.*, 2003), the CARDIA study (Pereira *et al.*, 2002), the Quebec Family Study (Jacqmain *et al.*, 2003) and the HERITAGE Family Study (Loos *et al.*, 2004) showed a significant inverse relationship between calcium consumption and body weight, the body mass index (BMI = body weight divided by the square of body length ($kg/m^2$)), body fat distribution, and the prevalence of obesity, respectively. The results were independent of whether calcium intake itself was estimated in the respective study or milk was taken as a measure of calcium intake.

Further evidence came from several clinical observational or controlled intervention studies, respectively, of which the primary focus was the calcium effect on bone mass (summarized by Davies *et al.*, 2000 and Heaney *et al.*, 2002), from studies relating nutrient intake to body composition and from some controlled intervention trials explicitly testing the calcium effect on body weight, fat and the efficacy of weight reduction diets (summarized by Scholz-Ahrens and Schrezenmeir, 2006). Whereas these studies consistently revealed that a high calcium intake in childhood and adulthood independent of the

© Woodhead Publishing Limited, 2010

Downloaded From Vet-eBooks.com

**Table 2.6** Effect of a 300 mg increment in regular calcium intake on body weight and fat

| Group | Change in body weight | Change in body fat | Reference |
|---|---|---|---|
| Children | | −1.0 kg | Carruth and Skinner (2001) |
| Young women | −2.5 kg | | Davies *et al.* (2000) |
| Adult women | −3.0 kg | | Zemel *et al.* (2000) |
| African-American men | | −4.9 kg | Zemel *et al.* (1990) |

calcium source (dairy, supplements, other foods) was positively associated with a lower body weight or BMI, respectively, as well as with a shift from fat to lean body mass and a slower age-dependent weight gain in midlife, other human studies failed to show positive effects of calcium on body weight and composition (Wosje and Kalkwarf, 2004; Bortolotti *et al.*, 2008) or positive calcium effects failed to reach statistical significance (Boon *et al.*, 2007).

A quantitative re-analysis of available data (Davies *et al.*, 2000) using simple bivariate and multivariate regression models revealed that calcium intake accounted for ~3% of the variation in BMI in young women and that every 100 mg increment in daily calcium intake decreased average BMI by 0.3 $kg/m^2$. Other studies showed somewhat more pronounced effects in adults (Table 2.6). But regarding population means these effects gain more weight. In young women, an increase in calcium intake from 500 to 1100 mg/d caused a drop in mean BMI by 1.8 $kg/m^2$ (−8%) but decreased the predicted prevalence of overweight/obesity (BMI $\geq$ 25 or 30 $kg/m^2$ respectively; WHO, 2000) substantially by 78% and 84%, respectively (Heaney *et al.*, 2002). Midlife weight gain decreased from 0.4 kg/year to 0.01 kg/year when comparing women below or at the 25th percentile of calcium intake with those at recommended calcium intake (Heaney, 2003), and one additional serving of calcium per day (300 mg) led to a decrease of 3.5–4.5% (−20%) of body fat in pre-school boys and girls (mean body fat ~18% or 21%, respectively) (Carruth and Skinner 2001).

IP Address: 129.132.208.100

*Mechanisms of the anti-obesity effect of calcium*

How does calcium work? Although the physiological or cellular basis for the changes in body weight and body fat has not been fully elucidated, several hypotheses have been developed. The oldest one starts from the chemical nature of calcium and postulates that the divalent cation calcium prevents intestinal absorption of part of the dietary fat and increases fecal lipid loss and sterol excretion by forming insoluble fatty acid soaps and bile salts in the gut (Denke *et al.*, 1993; Vaskonen, 2003). It is assumed that low calcium absorption (or bioavailability) in the intestine combined with a high calcium intake and thus calcium concentration in the intestine would be associated with less fat and energy intake (or rather availability to the body) and consequentially with a lower body weight.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

Another convincing idea developed by Zemel and co-workers (Zemel *et al.*, 2000) is based largely on experiments in obesity-prone (so-called agouti) mutant mice (Shi *et al.*, 2002; Zemel, 2002). According to this hypothesis, increased circulating $Ca^{2+}$ due to the consumption of relatively large amounts of dietary calcium decreases counter-regulatory serum concentrations of the calcitropic hormones PTH (parathyroid hormone) and calcitriol (1,25-dihydroxy-vitamin D3), which in turn down-regulates $Ca^{2+}$ influx into adipocytes and thereby intracellular calcium. Decreased adipocyte intracellular $Ca^{2+}$ stimulates lipolysis, fatty acid oxidation and, in some studies, the expression of uncoupling protein 2 and thereby thermogenesis. At the same time, lipogenic gene expression and fatty acid synthase activity are inhibited, but a contribution of *de novo* lipogenesis in the development of obesity in humans remains doubtful. All these effects result in decreased adipocyte lipid accumulation (Shi *et al.*, 2001), weight and body fat reduction and an overall shift of dietary energy from adipose tissue to lean body mass.

A third possible mechanism, which may weakly contribute to weight reduction as well, has recently been proposed (Ping-Delfos *et al.*, 2004). In a randomized, blind, controlled, crossover study in 11 overweight or obese subjects, a high calcium intake did not change hunger and satiety immediately after a meal, but did significantly reduce spontaneous food intake over the next 24 hours.

*Relevance of the anti-obesity effect of calcium*

Although the contribution of the different mechanisms to the overall anti-obesity effect of calcium is not clear, there is substantial evidence from animal and human studies that a high calcium intake decreases body weight and fat by a small but significant and relevant amount. However, the results of studies are contradictory in whether an increase of the calcium supply beyond recommendations of ~1000–1300 mg/day, which were set up in order to reduce the osteoporosis risk, would be able to increase the calcium effect on body weight further. Therefore, these effects can be achieved by milk and diets naturally rich in calcium and also by consuming calcium supplements and calcium-fortified food, paving the way for new nutritional concepts to fight obesity (Gerstner and de Vrese, 2007). However, calcium from milk and dairy products seems to be more effective than pure calcium preparations (Lin *et al.*, 2000; Zemel, 2002). This suggests that other milk constituents also contribute to the anti-obesity effect of milk.

IP Address: 129.132.208.100

### 2.4.3 Conjugated linoleic acid (CLA)

Dairy fat is the major natural source of conjugated linoleic acid (CLA), and CLA is a major dietary ruminant *trans*-fatty acid. Whereas studies in animals (hamsters) indicate that CLA may have beneficial effects on lipid parameters and body weight by reducing fat mass and increasing lean body mass (Larsen *et al.*, 2003; Bhattacharya *et al.*, 2006), early human trials lasting between 9 and 18

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

weeks and using CLA doses of about 3 to 3.5 g/d failed to confirm this (Zambell *et al.*, 2000; Whigham *et al.*, 2004) or showed only trends towards lower body fat mass, up to −1 kg (Malpuech-Brugère *et al.*, 2004; Taylor *et al.*, 2006). Animal studies suggest that the *trans*10,*cis*12-CLA isomer has a greater bodyweight-reducing potential than *cis*9,*trans*11-CLA, which is the dominant isomer in dairy fat. But a human study found comparable effects on body fat mass with both the *c*9,*t*11 and *t*10,*c*12 isomers (Malpuech-Brugère *et al.*, 2004). CLA was associated with increased resting energy expenditure and $\beta$-oxidation and a significant decrease of body weight or at least body fat and a concomitant increase in lean body mass (Nazare *et al.*, 2007). CLA did prevent an increase in body weight during winter, which was observed with safflower oil (linoleic acid rich) as control (Fig. 2.3; Watras *et al.*, 2007). The most pronounced effect of CLA was observed when combined with additional body weight-reducing measures, i.e. physical exercise, in young, normal-weight subjects: 1.8 g/d CLA plus a 90-minute vigorous run three times a week reduced in 12 weeks the body weight by −1.9 kg and the fat mass by −20% as compared to control subjects who did not receive CLA (Thom *et al.*, 2001). A meta-analysis in 2007 concluded that with 3.2 g/d CLA a moderate reduction of body fat can be achieved (Whigham *et al.*, 2007). The available data do not allow an estimate to be made of the amount of CLA required to achieve beneficial effects concerning weight and body fat reduction. This makes it difficult to give recommendations concerning an optimal milk fat consumption, or a reasonable CLA fortification of milk fat to be achieved either by modified feeding regimens of the cow or by adding isolated or chemically produced CLA.

IP Address: 129.132.208.100

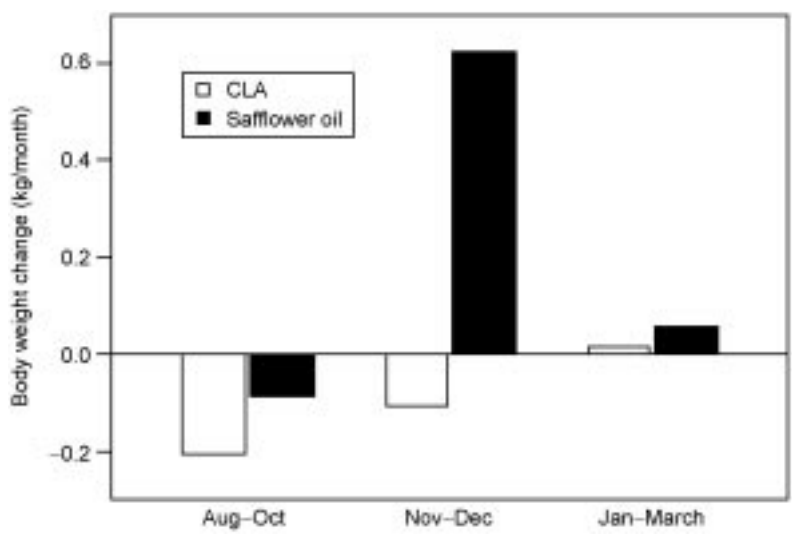



**Fig. 2.3** Effects of fatty acids and season (or holidays, respectively) on body weight in overweight subjects (18–44 years, $n = 40$). Results of giving 3.2 g/day CLA for 6 months in a randomized, double-blind, placebo-controlled study (Watras *et al.*, 2007, with permission of the authors).

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

### 2.4.4 Medium-chain fatty acids

Medium-chain fatty acids (MCFA) are naturally found in milk fat and coconut oil. Medium-chain triglycerides (MCT) are produced from coconut oil. Triglycerides with short- or medium-chain fatty acids have a higher proportion of carbon and hydrogen in the molecule and therefore a lower energy density than triglycerides with fatty acids with 16 or 18 C atoms (Smedman *et al.*, 1999). This is, however, not sufficient to explain the reductions of body fat mass induced by medium-chain fatty acids in several human studies (Tsuji *et al.*, 2001; Han *et al.*, 2007), and one must postulate an independent effect of MCFA.

Potential mechanisms are: (1) a preferential $\beta$-oxidation of medium-chain fatty acids rather than deposition in adipose tissue triglycerides; (2) an increase in resting glucose and lipid oxidation and in postprandial lipid oxidation and energy expenditure following administration of MCT or butterfat, respectively, to obese subjects or animals; and (3) down-regulation of adipogenic genes. Some of these findings add to the idea that medium-chain fatty acids may especially assist in the dietary management of existing obesity (Marten *et al.*, 2006; Rolland *et al.*, 2002).

### 2.4.5 Effects of milk (whey) protein and appetite-regulatory bioactive peptides

Milk (whey) protein has potential to contribute to the regulation of body weight by inducing satiety signals that affect both short-term and long-term food intake regulation. Although the role of individual whey proteins and peptides is not fully understood, it seems that whey protein reduces short-term food intake compared with placebo, carbohydrates and other proteins affecting satiation and satiety by the actions of whey protein fractions *per se*, bioactive peptides and/or amino acids released during digestion. Whey protein ingestion activates many components of the food intake regulatory system, it is insulinotropic, and peptides derived from whey affect the renin–angiotensin system, which may influence body weight (Sharma, 2008; Sarzani *et al.*, 2008). However, it is unclear whether measurable effects of whey on satiety and food intake and, therefore, potentially on obesity and its co-morbidities (hypertension, diabetes type II, hyper- and dyslipidemia) can be obtained through normal levels of consumption of dairy products. The effects described have been observed in short-term experiments and only with high amounts of whey protein (Luhovyy *et al.*, 2007; Anderson *et al.*, 2004).

### 2.4.6 Conclusions: is milk a fat burner?

Beside the relatively small energy density of milk and the possible association between high dairy consumption and a healthy lifestyle, some dairy constituents have direct, positive effects on body weight and body fat. Overall these effects are small and the study outcomes are not sufficiently consistent, such that one cannot name milk, calcium or CLA as 'fat burners'. The individual consumer of

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

milk and dairy products will, therefore, experience the beneficial effect on body weight and body fat at most as an augmentation of other dietary measures and changes in lifestyle (more physical activity, less calories). If they are confirmed, they are, however, relevant to the general population.

## 2.5 Effects of natural and added milk constituents, particularly pro- and prebiotics, on gut health

### 2.5.1 General aspects: gut, intestinal microbiota and immunity

The functionality and health of the gut are largely determined by three components, which are closely intertwined:

- The gut itself (lumen, mucosa) including the gut-associated immune system (GALT)
- The indigenous microbiota[1] of the gut, which include about $10^{14}$ bacteria and other microorganisms (more cells than in the entire human body)
- Nutrients, fibers and other natural or added food components such as, for example, probiotic bacteria or prebiotic carbohydrates. They can have an impact on gut health as well as on the health of the consumer in a positive or negative way.

The intestinal microbiota plays an important role in the defense against infections by viruses, bacteria or fungi, for which the gut is the main entry point (Marteau *et al.*, 2001). The protective effect is mediated by:

- Direct inhibition of pathogenic microorganisms, by blocking potential binding sites for bacteria at the mucosa surface, by competing for nutrients and by releasing bacteriostatic, bactericidal or fungicidal substances
- Indirect mechanisms, particularly by influencing the development of the immune system after birth and later by stimulating or rather modulating the gut-associated immune system and thus modulating the immune defense of other compartments of the body.

IP Address: 129.132.208.100

The gastrointestinal microbiota of adults comprise 400–500 (known) different bacterial strains, and as such form a highly complex and balanced ecosystem. The number of bacteria increases from $10^3$–$10^5$/g contents in the stomach up to $10^8$–$10^{11}$/g intestinal content in the colon and rectum. Once the intestinal microbiota has been established, the composition of an 'adult' gut microbiota remains remarkably stable over many decades (Mitsuoka, 1992).

Options to modify it in a 'positive' way are through the administration of health-promoting 'probiotic' bacteria (Chermesh and Eliakim, 2006) or by so-called prebiotics, i.e. carbohydrates which specifically promote the growth and proliferation of health-promoting bacteria. Furthermore, some natural

1. In modern literature the term 'microbiota' is used instead of 'microflora' or 'intestinal flora', because bacteria and fungi are not plants.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

ingredients of milk and fermented milk products (calcium, lactic acid and short-chain fatty acids, lactoferrin, immunoglobulins and other whey proteins, as well as certain bioactive peptides released from milk proteins during fermentation and digestion of milk) do reduce the infection risk, have an immuno-modulating or immuno-stimulating effect, or promote gut health through other mechanisms.

### 2.5.2 Beneficial effects of natural constituents of milk and milk products on gut health

Certain natural constituents of milk and milk products may contribute to the prevention, delayed onset or alleviation of certain disorders and diseases of the gastrointestinal tract, e.g. colonic cancer, increased release of putrefactive substances of bacterial and host metabolism, several types of diarrhea, infections and/or inflammation of the gastrointestinal tract or disturbed immune functions (Table 2.7). While protective effects of mothers' milk against infections of the gastrointestinal tract of newborns and the maturation of their gut-associated immune system are scientifically accepted, the relevance of the respective components of cows' milk for the gut health of adult subjects is unclear. Therefore no recommendations have been made to improve gut-protective properties of milk by increasing its content of protective lipids through modified feeding or by fortifying milk with calcium, certain whey proteins or bioactive peptides. However, more extensive randomized, controlled clinical studies are necessary.

IP Address: 129.132.208.100

**Table 2.7** Gut protective (natural) constituents of milk and dairy products

| Component | Mechanism(s) | Health effects |
|---|---|---|
| Calcium | Reduction of secondary bile acids | Colorectal cancer ↓ |
| | Activity against pathogenic bacteria | Intestinal infection ↓ |
| Lactic acid, fatty acids | pH Reduction | Bacterial infections (*H. pylori*) ↓ |
| | Stimulation of carbohydrate-utilizing bacteria instead of a more putrefactive intestinal microbiota | Putrefactive metabolites ↓ |
| | | Cancer ↓ |
| $\omega$3 Fatty acids | Anti-inflammatory effects | Inflammation ↓ |
| Whey proteins | | Improvement of immune functions |
| Lactoferrin | Activity against pathogenic bacteria | Infections ↓, inflammation ↓ |
| Immunoglobulins | Immunostimulation | Infections ↓ |
| Bioactive peptides released from milk proteins by fermentation or digestion | Immunostimulation | Infections ↓ |
| | Increased intestinal motility | |

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

### 2.5.3 Fermented milk products, probiotics and gastrointestinal complaints and diseases: health effects and mechanisms

The addition of probiotic bacteria to (fermented) milk products has become the most successful approach to create milk products with increased gut-protective properties (Chermesh and Eliakim, 2006). Probiotics are defined as 'live micro-organisms, which, when administered in adequate amounts, confer a (scientifically proven) health benefit on the host' (FAO/WHO, 2001). Probiotic foods contain probiotic bacteria at effective concentrations and are the almost ideal functional foods. This means that their consumption leads to a proven positive health effect by improving body functions (immune system, intestinal activity) beyond its nutritional value (Table 2.8).

Suggested, but unconfirmed, mechanisms for probiotic effects include:

- Direct effects on the intestinal microbiota, e.g. reduction of the intestinal pH, production of bactericidal substances (e.g. organic acids, $H_2O_2$ and bacteriocines), agglutination of pathogenic microorganisms, strengthening barrier function of the intestinal mucosa, competition for fermentable substrates or receptors on the cellular surface of the mucosa (Gill *et al.*, 2001)
- Release of gut-protective compounds (arginine, glutamine, short-chain fatty acids, CLA) and absorption and metabolization of potentially pathogenic, toxic or carcinogenic metabolites and enzymes (Fonden *et al.*, 2000; Ouwehand *et al.*, 2002)

IP Address: 129.132.208.100

**Table 2.8** Health effects of probiotics

| Assured effects | Potential effects | Effects outside the intestine |
|---|---|---|
| Survival/modulation of intestinal microbiota | Improvement of the balance of the intestinal microbiota | Inhibition of the gastric pathogen *Helicobacter pylori* |
| Increase of lactose digestion, reduction of complaints of lactose intolerance | Normalization of intestinal movements | Prevention or alleviation of bacterial or fungal infections of the urogenital tract and the oral cavity |
| Less frequent and shorter episodes of rotavirus- or antibiotics-induced diarrhea | Less irregular, unspecific gastrointestinal complaints Use during inflammatory bowel disease | Prevention or alleviation of viral and bacterial infections of the upper respiratory tract |
| Decrease of health-impairing metabolites and cancer-promoting enzymes in the intestine | Protection against (intestinal) cancer | Immunomodulation Allergic/atopic diseases ↓ |
| Immunomodulation | Prevention or alleviation of viral, bacterial or fungal infections of the intestinal tract | Hypercholesterolemic effect |

© Woodhead Publishing Limited, 2010

Downloaded From Vet-eBooks.com

- Modulation of immunologic mechanisms (Walker, 2000)
- Stimulation of intestinal motility and mucus production.

*Evidence for beneficial effects of probiotics and probiotic milk products on lactose intolerance*

Without any doubt, the most thoroughly investigated health-related effect of fermented milk products and in particular of yogurt is the improvement of lactose digestion. The disaccharide lactose is present as a natural constituent only in milk and dairy products. In the gastrointestinal tract, lactose is hydrolyzed in the small intestine by the enzyme $\beta$-galactosidase (lactase) into glucose and galactose. These components are absorbed. In most people worldwide lactase activity decreases between the ages of approximately 4 and 20 years, depending on their ethnic background or country of origin (de Vrese *et al.*, 2001).

The prevalence of the so-called lactose malabsorption (or, better, lactose maldigestion) in adults is about 60% in some parts of southern and eastern Europe, 70% in Africa and South America and almost 100% in south-east Asia, but only 3–15% in northern and mid-Europeans and their descendants in North America, Australia and New Zealand (Scrimshaw and Murray, 1988). The reason for this low frequency of lactose malabsorption has been further elucidated by work of Finnish, US and German geneticists who found out that ~5000 years ago a lactase persistency mutation occurred in Europe around the Baltic sea (Burger *et al.*, 2007). The frequency of lactase persistency then increased from 0 to >70% within ~3000 years and is therefore one of the fastest evolutionary processes known. Similar mutations may have occurred at various times in different regions of the earth, but did not prevail for various reasons.

IP Address: 129.132.208.100

Depending on the composition of the intestinal microbiota and/or the individual sensitivity to pain, milk or (better) lactose intake leads, in a proportion of lactose malabsorbers (in Germany ~50%), to symptoms of bloating, flatulence, cramps, abdominal pain and diarrhea, which are caused by the osmotic activity of undigested lactose as well as by gaseous fermentation products in the large intestine. This phenomenon is called (primary) lactose intolerance.

It is generally recommended to these persons that they abstain from the consumption of milk and dairy products. However, most lactose-intolerant persons are able to digest small amounts of milk (approximately 200 ml), in particular if consumed within a mixed meal. They can consume dairy products from which lactose is removed by lactose hydrolysis or chromatographically. Ripened cheese varieties contain no or only small amounts of lactose.

Furthermore, most lactose maldigesters tolerate fermented milk products much better than unfermented milk, because these products contain live bacteria with active microbial $\beta$-galactosidase that survives the passage through the stomach. This enzyme is finally liberated in the small intestine where it supports lactose hydrolysis (de Vrese *et al.*, 1997). This is not a specific probiotic effect: conventional yogurt is mostly more effective than probiotic

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

products (de Vrese *et al.*, 2001), because many probiotic bacteria show either a lower $\beta$-galactosidase activity than the conventional yogurt starter, or, due to their high stability to acid and bile salts, do not release most of their $\beta$-galactosidase in the small intestine. Independent from such effects on lactose maldigestion, probiotics seem to reduce gastrointestinal complaints like flatulence or diarrhea, possibly by their impact on the intestinal microbiota (Zhong *et al.*, 2004).

*Evidence for beneficial effects of probiotics and probiotic milk products on gastrointestinal infections and diarrhea*

Enteric infections caused by viruses (rotavirus) or bacteria are still a major problem and are responsible for about 1.8 million deaths each year, particularly in hospitalized children and in developing countries. Probiotics can potentially provide an important means to reduce these problems; indeed, at the beginning of the twentieth century conventional yogurt was developed as an inexpensive means to fight (frequently deadly) diarrhea in small children and was sold in pharmacies. Beneficial effects, such as decreased frequency of infections, shortening of the duration of episodes by 1–1.5 days, less shedding of rotaviruses or an increase in the production of specific antibodies, have been demonstrated for *Lactobacillus rhamnosus* GG, *L. casei* Shirota, *L. reuteri*, *Bifidobacterium animalis* ssp. *lactis* Bb-12 and a number of other probiotic strains in clinical studies fulfilling scientific requirements, whereas inhibition of growth and adhesion of a range of enteropathogenic bacteria such as *Salmonella* has been demonstrated mainly *in vitro* and in animal studies (Sazawal *et al.*, 2006; Huang *et al.*, 2002; Allan *et al.*, 2004; de Vrese and Marteau, 2007; Majamaa *et al.*, 1995; Krammer *et al.*, 2006).

In three studies in young healthy children attending daycare centers, administration of conventional yogurt or probiotic milk products reduced the number of days of absence from daycare due to gastrointestinal and respiratory tract infections between 16 and 46%, respectively (Pedone *et al.*, 1999, 2000; Hatakka *et al.*, 2001; Weizman *et al.*, 2005).

Investigations of probiotic effects on traveler's diarrhea showed inconsistent results (Hilton *et al.*, 1997; Katelaris *et al.*, 1995). This may result from differences between probiotic strains, the countries traveled in and the local microbiota, the eating habits of the travelers, or the means of administration of the probiotic (i.e. before or during travel, as a capsule or as a fermented milk product).

Administration of certain probiotic strains before and during antibiotic treatment in most studies reduced the frequency and/or duration of episodes of antibiotic-associated diarrhea and the severity of symptoms (Cremonini *et al.*, 2002; McFarland, 2006; Szajewska *et al.*, 2006), although there are many reports demonstrating no effects. Administration of *B. animalis* ssp. *lactis* and *Lactobacillus acidophilus* four weeks before and during a *Helicobacter pylori* eradication therapy led to significantly fewer episodes of diarrhea (7% versus 22% of the subjects) compared with the placebo group (de Vrese 2003; Fig. 2.4).

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

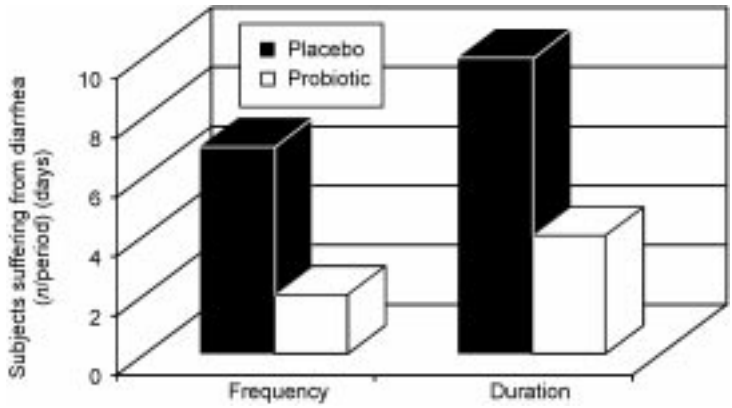



**Fig. 2.4** Effect of probiotic lactobacilli and bifidobacteria (*L. acidophilus* LA5 + *B. animalis* ssp. *lactis* Bb12) on frequency and duration of diarrhea due to antibiotics treatment of *Helicobacter pylori* infected subjects (de Vrese, 2003).

In the same study, not only the probiotics but even unfermented milk that had been acidified with lactic acid significantly reduced the activity of *H. pylori* in the stomach.

In some cases antibiotic treatment may result in life-threatening pseudomembranous colitis, which is associated with an abundance of anaerobic toxigenic bacteria (e.g. strains of *Clostridium difficile*). Application of probiotics also significantly decreased the number of relapses in successfully treated *C. difficile* infections (Dendukuri *et al.*, 2005).

IP Address: 129.132.208.100

*Constipation*

The assumption that probiotics are able to alleviate constipation (i.e. slow gastrointestinal transit, excessive stool hardness, difficulties in passing stool) relies more frequently on anecdotal reports rather than on controlled clinical trials. An unclear definition of constipation, a lack of appropriate end-point markers, insufficiently detailed symptom questionnaires, and unsatisfactory recording of the health and well-being of the subjects have resulted in numerous confusing and contradictory results. Recent controlled clinical studies showed that administration of certain probiotic strains belonging to *L. casei* (Koebnick *et al.*, 2003) and *B. animalis* (Bouvier *et al.*, 2001) reduced gastrointestinal transit time, and, very recently, a probiotic fermented milk product was introduced in the market with the claim to fight constipation. Nevertheless, more controlled clinical studies with clearly defined end-point markers and sufficient numbers of participants are strongly recommended.

*Evidence for beneficial effects of probiotics and probiotic milk products on inflammatory bowel diseases (IBD) and irritable bowel syndrome (IBS)*

Inflammatory bowel diseases, such as Crohn's disease, ulcerative colitis and pouchitis, as well as irritable bowel syndrome, may in part be caused or

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

aggravated by alterations in the gut microbiota, including infection (Shanahan, 2000; Fanigliulo *et al.*, 2006), and the stimulation of pro-inflammatory immunological mechanisms seems to play a crucial role in inflammatory diseases of the intestine (Dotan and Rachmilewitz, 2005). Therefore numerous efforts have been undertaken to improve the health and well-being of affected patients by the administration of probiotics with anti-inflammatory properties and a demonstrated positive impact on the intestinal microbiota. Whereas some studies showed a positive modulation of the intestinal microbiota and the alleviation of symptoms (Ishikawa *et al.*, 2003; Gionchetti *et al.*, 2006; Kajander and Korpela, 2006), other studies failed to do so (Marteau *et al.*, 2006), and further investigations are required to move from hopeful findings to conclusive results. These studies are in the first line of medical interest and are usually carried out with probiotic preparations, seldom with probiotic milk products, and it is difficult to convey positive outcomes to the consumer in an understandable and promotionally effective manner.

*Evidence for beneficial effects of probiotics and probiotic milk products on cancer*

There is, at best, preliminary evidence that probiotic microorganisms can prevent or delay the onset of certain cancers (de Roos and Katan, 2000), in particular colonic cancer, the most frequent cancer of the intestinal tract in Western industrial nations (Rafter, 1995). This evidence stems primarily from *in vitro* and animal studies, in which (1) inhibition of tumor growth and proliferation of tumor cells by glycopeptides and cytotoxic metabolites of lactobacilli, (2) antimutagenic properties of probiotics and probiotic milk products, (3) the strengthening of the immune system, and (4) the reduction of (pro)carcinogenic, mutagenic and genotoxic substances (aflatoxins, nitrosamines) and cancer-promoting enzymes (nitroreductase, azoreductase, $\beta$-glucuronidase) in the colon have been observed (Ouwehand *et al.*, 2002; Hayatsu and Hayatsu, 1993). The latter effect may result from modifications of the gut microbiota, a decrease in intestinal pH, chemical modification and absorption by the bacteria.

However, more epidemiological data and more and longer-lasting studies in humans using universally accepted markers for cancer are necessary before definitive clinical conclusions regarding the efficacy of probiotics in cancer prevention can be drawn.

IP Address: 129.132.208.100

### 2.5.4 Prebiotics

As well as by ingestion of probiotic bacteria, the intestinal microbiota, the intestinal milieu and the gut-associated immune system can be affected positively by so-called prebiotics: 'a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health' (Gibson and Roberfroid, 1995) or, according to a revisited definition, 'selectively fermented [food] ingredients that allow specific changes, both in the composition

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

**Table 2.9** Gut-associated health effects of prebiotics

| Health effect | Evidence |
|---|---|
| Bifidobacteria↑, short chain fatty acids↑, stool pH↓ (Cherbut, 2002; Cummings and Macfarlane, 2002) | Good |
| Gastrointestinal infections↓, inflammatory bowel syndrome, irritable colon ↓ (Knol *et al.*, 2005; Colecchia *et al.*, 2006; Lindsay *et al.*, 2006) | Preliminary |
| Diarrhea↓ (Lewis *et al.*, 2005; Cummings *et al.*, 2001) | Preliminary |
| Constipation↓ (Cherbut, 2002) | Preliminary |
| Colonic cancer↓ (Rafter *et al.*, 2007) | Preliminary |
| Calcium absorption from the gut↑ (Scholz-Ahrens *et al.*, 2002; Abrams *et al.*, 2005) | Good |
| Stool microbiota more similar to that of breast-fed babies (Knol *et al.*, 2005; Fanaro *et al.*, 2005) | Good |
| Positive health effects of a synergistic ('symbiotic') combination of pro- and prebiotics (Bartosch *et al.*, 2005) | Preliminary, many poor studies |

and/or activity in the gastrointestinal microbiota that confers benefits upon host well being and health' (Roberfroid, 2007). An overview of gut-associated prebiotic health effects is given in Table 2.9.

All known prebiotics used by the food industry for technological reasons (modification of the viscosity, emulsification capacity, gel formation, freezing point and color of foods) or to improve health properties of a food are bifidogenic carbohydrates, particularly inulin, fructo-oligosaccharides (oligofructose, OF) prepared from these carbohydrates by hydrolysis, and short-chain galacto-oligosaccharides (GOS, TOS), which are synthesized via transgalactosylation of lactose by using $\beta$-galactosidase. Inulin is a starch-like mixture of fructo-oligosaccharides and polysaccharides, and is a component of numerous food plants such as chicory, onions, leeks, Jerusalem artichokes, artichokes, bananas and cereals.

Unlike mare's or mother's milk, which are high in very complex galacto-oligosaccharides, cow's milk naturally contains no or only irrelevant amounts of prebiotic carbohydrates. Therefore inulin or oligofructose are added to conventional or probiotic (fermented) milk products; the latter are often also called 'symbiotic'. To prevent symptoms like flatulence, bloating, abdominal pain or diarrhea even in very sensitive subjects, frequently just small amounts of inulin or oligofructose (as little as 2 g per meal) are added, despite the fact that a health effect from such a small quantity has not been demonstrated.

### 2.5.5 Safety of pro- and prebiotics

The safety aspects of probiotic (fermented) milk products will be covered very shortly, because (probiotic) lactobacilli and bifidobacteria used for food manu-

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

facturing are generally accepted as safe (Boriello *et al.*, 2003). The strongest proof of their safety is the century-long use of lactic acid bacteria in the food area without negative consequences, as well as the absence of risk factors from these bacteria. This is not changed by the results of a recently published study, in which administration of a probiotic multistrain preparation to critically ill patients ($n = 296$) with predicted severe acute pancreatitis was associated with a significantly higher mortality in the group given probiotics (16%) compared with the control group (6%) (Besselink *et al.*, 2008). This has, however, nothing to do with the customary consumption of food probiotics by 'normal', not immuno-compromised, consumers with an intact intestinal barrier function, so that one can still assume that the probiotic bacteria used in foods are safe.

Nevertheless, lactobacilli and bifidobacteria, although apathogenic, are involved in ~0.1% of cases of sepsis, meningitis and endocarditis. In almost all cases, however, the bacteria stemmed from the indigenous gut microbiota, and translocation and infections were often facilitated by a weakened body defense. Only a handful of cases have been published, where (external) probiotics used for food manufacture were involved.

Prebiotics, although regarded as safe, may lead to gastrointestinal symptoms such as flatulence, bloating, cramps, abdominal pain or diarrhea in a certain number of consumers. Whereas most of them tolerate up to 10–20 g inulin or oligofructose, some extremely sensitive subjects respond to as little as ~2 g. Galacto-oligosaccharides are better tolerated.

IP Address: 129.132.208.100

### 2.5.6 Improving milk products for gut health: quality aspects

By definition, the quality of probiotic (fermented) milk products here means only their health promoting and maintaining properties. To be claimed, such positive health effects must be proven in more than one independent, placebo-controlled, randomized, double-blind clinical study with a sufficient number of subjects and must be published in peer-reviewed scientific journals. Generally, probiotic effects are strain-specific. Therefore bacterial cultures used for manufacturing probiotic milk products must be stable, unequivocally identifiable and clearly defined with modern molecular biological verification techniques. A strain allocation based on phenotypic characteristics alone is not sufficient.

Furthermore, probiotic effects are target group- and matrix-specific. This means that the effects of probiotic microorganisms on study participants may vary with their indigenous intestinal microbiota, their age, health and gender, diet, residence and environment (e.g. rural or urban) and, therefore, whether probiotic bacteria are ingested either within a fermented milk product together with other starter organisms and possible fruit admixture or as a pure probiotic bacterial powder or capsule.

Last but not least, probiotic bacteria should be technologically suited, i.e. they need to survive in the product and during minimum shelf-life in sufficient concentration and must not impair product quality. The term 'sufficient number' is used when a regular daily serving contains $10^8$ probiotic bacteria or ~$10^6$ cfu/g

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

fermented milk product. In any case, a probiotic product should guarantee the ingestion of that number of probiotic microorganisms at the end of its shelf-life. This is the number of cells applied in the studies substantiating their health effects.

A series of approaches to design probiotic milk products with (enhanced) gut health-promoting properties have been developed. These include:

1. Searching for 'new' probiotic strains with improved or new health effects, or proof of new effects of probiotic cultures already on the food market. In all cases the improvements must be shown in clinical studies that comply with the above-mentioned criteria.
2. Selection of probiotic bacteria that are sufficiently acid- and bile salt stable and resistant against digestive enzymes, in order to ensure survival of a sufficient number of cells during gastrointestinal passage. (Due to the high costs involved, tasks 1 and 2 are usually conducted by the company providing the cultures.)
3. Introducing a high number of probiotic bacteria in the product and improving survival in the product during its shelf-life by selecting strains with good stability against acids and sufficient oxygen tolerance, as well as selection of weakly acidifying fermentation cultures, which in part can also act to protect against oxygen consumption (*S. thermophilus* is frequently used).
4. A further option to improve survival in the product during the gastrointestinal passage and to afford a targeted release of the probiotics in the intestine is the encapsulation of active or (freeze)-dried probiotic bacteria in small (0.1–50 $\mu$m) microcapsules or microparticles prepared from various materials (e.g. alginate or food-grade hard fats).
5. Most probiotic foods on the market are yogurt-like, set or fluid fermented milk products. Therefore it may be wise to develop probiotic alternatives for people who do not like yogurt or other fermented milk products. Examples are shown in Table 2.10.

IP Address: 129.132.208.100

While for 'probiotic' cheese and ice-cream at least a long-term survival of probiotic bacteria in the product has been shown, health-promoting properties of these milk (and non-milk) products were only deduced from the use of acknowledged probiotic strains and (at least for cheese) from the recovery of the added probiotics in the feces. As yet, no clinical studies have been performed that prove the health-promoting effects of these foods. It is therefore to be expected that fluid or set yogurt-like products with probiotic bacteria will remain the only relevant probiotic food, at least for the near future.

### 2.5.7 Conclusions: contradictory or negative study results – proof of a lack of pro-, pre- or symbiotic milk products?

Despite the unquestionable market success of functional fermented (and non-fermented) milk products with probiotic bacteria and/or prebiotic carbohydrates, despite the exponentially increasing publications on probiotics, and despite

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

**Table 2.10** Examples of (potential) non-yogurt probiotic food

| Product | Stage of development[a] | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Whey drinks, buttermilk, kefir, dahi | + | + | +/− | − | − |
| Fresh cheese (cottage cheese, quarg), ripened cheese | + | + | +/− | +/− | − |
| Ice cream, frozen yogurt | + | + | +/− | +/− | − |
| Sweets, cookies, cake chocolate, milk cream, cake | − | +/− | − | − | − |
| Cereals (muesli) plus lyophilized probiotics | + | (+) | − | − | − |
| Lyophilized milk powder | + | + | − | − | − |
| Probiotic raw sausages | + | + | − | − | − |
| Vegetables ('sauerkraut', kimchi) | + | + | +/− | − | − |
| Products containing microencapsulated (lyophilized) bacteria | + | + | +/− | +/− | − |

[a] 1 = Sufficient numbers of bacteria in the products are possible
2 = Survival in the product is sure
3 = Survival during GI passage has been proven
4 = Use of established probiotics
5 = Proof of probiotic health effects in clinical studies.

increasing understanding of the underlying probiotic mechanisms of effect and the connection between intestinal flora and the probiotics and prebiotics added and their relevance for immunological and non-immunological processes of defense, it is frequently argued that pro- and prebiotics have no health effects – mostly with reference to negative or contradictory study outcomes. These objections disregard the fact that clinical studies yield statistically significant results and, due to strain, matrix and target group specificity of pro- and symbiotic effects of such products, can have variable effects in different (age) groups of the population or in different individuals. This means that each consumer needs to test by himself or herself whether and to what extent he or she profits from pro- and prebiotics. There is definitely no health risk from pro- and prebiotic foods whatsoever.

IP Address: 129.132.208.100

## 2.6 Sources of further information and advice

The scientific literature on the topics of milk, dairy products (including pro- and prebiotic foods) and milk constituents and their effects on bone and teeth health, overweight and obesity and lipid metabolism, is so extensive, with many monographs, that it is virtually impossible to select a short list of representative books or review articles. Simply as examples without rating, we list the following books and review articles, which give a comprehensive overview on the topics discussed in this chapter: 'Diet, nutrition, and bone health' (Cashman, 2007), 'Nutrition and bone growth and development' (Prentice *et al.*, 2006); 'Milk and the metabolic syndrome' (Pfeuffer and Schrezenmeir, 2007), *Food, Diet and Obesity* (Mela, 2005), and 'Probiotics, prebiotics and symbiotics' (de

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

Vrese and Schrezenmeir, 2008). An overview of all aspects of functional fermented food is given by Farnworth (2008).

## 2.7 References


ABRAMS, SA, GRIFFIN, IJ, HAWTHORNE, KM, LIANG, L, GUNN, SK, DARLINGTON, G, ELLIS, KJ (2005), 'A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents'. *American Journal of Clinical Nutrition*, vol. 82, no. 2, pp. 471–6.

ADOLPHI, B, SCHOLZ-AHRENS, KE, DE VRESE, M, AÇIL, Y, LAUE, C, SCHREZENMEIR, J (2009), 'Short-term effect of bedtime consumption of fermented milk supplemented with calcium, inulin-type fructans and caseinphosphopeptides on bone metabolism in healthy, postmenopausal women', *European Journal of Nutrition*, vol. 48, no. 1, pp. 45–53.

AGERHOL-LARSEN, L, BELL, ML, GRUNWALD, GK, ASTRUP, A (2000), 'The effect of a probiotic milk product on plasma cholesterol: a meta-analysis of short-term intervention studies', *European Journal of Clinical Nutrition*, vol. 54, pp. 856–60.

AIHARA, K, KAJIMOTO, O, HIRATA, H, TAKAHASHI, R, NAKAMURA, Y (2005), 'Effect of powdered fermented milk with *Lactobacillus helveticus* on subjects with high-normal blood pressure or mild hypertension', *Journal of the American College of Nutrition*, vol. 24, pp. 257–65.

ALBERTSON, AM, GOOD, CK, HOLSCHUH, NM, ELDRIDGE, EL (2003), 'The relationship between dietary calcium intake and body mass index in adult women: data from the Continuing Survey of Food Intake by Individuals 1994–96', *FASEB J*, vol. 17, p. A289.

ALLAN, SJ, OKOKO, B, MARTINEZ, E, GREGORIO, G, DANS, LF (2004), 'Probiotics for treating infectious diarrhoea', *Cochrane Database of Systematic Reviews*, vol. 2, CD003048.

ANDERSON, GH, TECIMER, SN, SHAH, D, ZAFAR, TA (2004), 'Protein source, quantity, and time of consumption determine the effect of proteins on short-term food intake in young men', *Journal of Nutrition*, vol. 134, no. 11, pp. 3011–15.

AOE, S, TOBA, Y, YAMAMURA, J, KAWAKAMI, H, YAHIRO, M, KUMEGAWA, M, ITABASHI, A, TAKADA, Y (2001), 'Controlled trial of the effects of milk basic protein (MBP) supplementation on bone metabolism in healthy adult women', *Bioscience, Biotechnology, and Biochemistry*, vol. 65, pp. 913–18.

AOE, S, KOYAMA, T, TOBA, Y, ITABASHI, A, TAKADA, Y (2005), 'A controlled trial of the effect of milk basic protein (MBP) supplementation on bone metabolism in healthy menopausal women', *Osteoporosis International,* vol. 16, pp. 2123–8.

APPEL, LJ, MOORE, TJ, OBARZANEK, E, VOLLMER, WM, SVETKEY, LP, SACKS, FM, BRAY, GA, VOGT, TM, CUTLER, JA, WINDHAUSER, MM, LIN, PH, KARANJA, N, DASH COLLABORATIVE RESEARCH GROUP (1997), 'A clinical trial of the effects of dietary patterns on blood pressure', *New England Journal of Medicine*, vol. 336, pp. 1117–24.

APPEL, LJ, MILLER, ER III, JEE, SH, STOLZENBERG-SOLOMON, R, LIN, PH, ERLINGER, T, NADEAU, MR, SELHUB, J (2000), 'Effect of dietary patterns on serum homocysteine: Results of a randomized, controlled feeding study', *Circulation*, vol. 102, pp. 852–7.

ARD, JD, GRAMBOW, SC, LIU, D, SLENTZ, CA, KRAUS, WE, SVETKEY, LP, PREMIER STUDY GROUP (2004), 'The effect of the PREMIER interventions on insulin sensitivity', *Diabetes Care*, vol. 27, pp. 340–7.


IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

ATKINSON, SA, MCCABE, GP, WEAVER, CM, ABRAMS, SA, O'BRIEN, KO (2008), 'Are current calcium recommendations for adolescents higher than needed to achieve optimal peak bone mass? The controversy', *Journal of Nutrition*, vol. 138, pp. 1182–6.

ATTAR-BASHI, NM, WEISINGER, RS, BEGG, DP, LI, D, SINCLAIR, AJ (2007), 'Failure of conjugated linoleic acid supplementation to enhance biosynthesis of docosahexaenoic acid from alpha-linolenic acid in healthy human volunteers', *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, vol. 76, pp. 121–30.

BARTOSCH, S, WOODMANSEY, EJ, PATERSON, JC, MCMURDO, ME, MACFARLANE, GT (2005), 'Microbiological effects of consuming a synbiotic containing *Bifidobacterium bifidum*, *Bifidobacterium lactis*, and oligofructose in elderly persons, determined by real-time polymerase chain reaction and counting of viable bacteria', *Clinical Infectious Diseases*, vol. 40, no. 1, pp. 28–37.

BELOBRAJDIC, DP, MCINTOSH, GH, OWENS, JA (2004), 'A high-whey-protein diet reduces body weight gain and alters insulin sensitivity relative to red meat in Wistar rats', *Journal of Nutrition*, vol. 134, pp. 1454–8.

BENABLE, JE, MARTINEZ-MALDONADO, M (1991), 'Renal effect of dietary protein excess and deprivation', *Seminars in Nephrology*, pp. 76–85.

BENITO, P, CABALLERO, J, MORENO, J, GUTIÉRREZ-ALCÁNTARA, C, MUÑOZ, C, ROJO, G, GARCIA, S, SORIGUER, FC (2006) 'Effects of milk enriched with omega-3 fatty acid, oleic acid and folic acid in patients with metabolic syndrome', *Clinical Nutrition*, vol. 25, pp. 581–7.

BESSELINK, MG, VAN SANTVOORT, HC, BUSKENS, E, *ET AL.*, ACUTE PANCREATITIS STUDY GROUP (2008), 'Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial', *Lancet*, vol. 371, pp. 651–9.

BHATTACHARYA, A, BANU, J, RAHMAN, M, CAUSEY, J, FERNANDES, G (2006), 'Biological effects of conjugated linoleic acids in health and disease', *Journal of Nutritional Biochemistry*, vol. 17, pp. 789–810.

BIONG, AS, MULLER, H, SELJEFLOT, I, VEIEROD, MB, PEDERSEN, JI (2004), 'A comparison of the effects of cheese and butter on serum lipids, haemostatic variables and homocysteine', *British Journal of Nutrition*, vol. 92, pp. 791–7.

BLOUET, C, MARIOTTI, F, MIKOGAMI, T, TOME, D, HUNEAU, JF (2007), 'Meal cysteine improves postprandial glucose control in rats fed a high-sucrose meal', *Journal of Nutritional Biochemistry*, vol. 18, pp. 519–24.

BOON, N, HUL, GB, STEGEN, JH, SLUIJSMANS, WE, VALLE, C, LANGIN, D, VIGUERIE, N, SARIS, WH (2007), 'An intervention study of the effects of calcium intake on faecal fat excretion, energy metabolism and adipose tissue mRNA expression of lipid-metabolism related proteins', *International Journal of Obesity* (London), vol. 31, no. 11, pp. 1704–12.

BORIELLO, SP, HAMMES, WP, HOLZAPFEL, W, MARTEAU, P, SCHREZENMEIR, J, VAARA, M, VALTONEN, V (2003), 'Safety of probiotics that contain lactobacilli or bifidobacteria', *Clinical Infectious Diseases*, vol. 36, pp. 775–80.

BORTOLOTTI, M, RUDELLE, S, SCHNEITER, P, VIDAL, H, LOIZON, E, TAPPY, L, ACHESON, KJ (2008), 'Dairy calcium supplementation in overweight or obese persons: its effect on markers of fat metabolism', *American Journal of Clinical Nutrition*, vol. 88, no. 4, pp. 877–85.

BOTTAZZI, V (1983), 'Food and feed production with microorganisms', *Biotechnology*, vol. 5, pp. 315–63.

BOUHALLAB, S, BOUGLÉ, D (2004), 'Biopeptides of milk: caseinophosphopeptides and mineral bioavailability', *Reproduction, Nutrition, Development*, vol. 44, no. 5, pp. 493–8.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

BOUVIER, M, MEANCE, S, BOULEY, C, BERTA, J-L, GRIMAUD, J-C (2001), 'Effects of consumption of a milk fermented by the probiotic strain *Bifidobacterium animalis* DN-173 010 on colonic transit times in healthy humans', *Bioscience and Microflora*, vol. 20, pp. 43–8.

BOWEN, WH, LAWRENCE, RA (2005), 'Comparison of the cariogenicity of cola, honey, cow milk, human milk, and sucrose', *Pediatrics*, vol. 116, pp. 921–6.

BRONNER, F, PANSU, D (1999), 'Nutritional aspects of calcium absorption', *Journal of Nutrition*, vol. 129, no. 1, pp. 9–12.

BUONOPANE, GJ, KILARA, A, SMITH, JS, MCCARTHY, RD (1992), 'Effect of skim milk supplementation on blood cholesterol concentration, blood pressure and triglycerides in a free-living human population', *Journal of the American College of Nutrition*, vol. 11, pp. 56–67.

BURGE, R, DAWSON-HUGHES, B, SOLOMON, DH, *ET AL.* (2007), 'Incidence and economic burden of osteoporosis-related fractures in the United States, 2005–2025', *Journal of Bone and Mineral Research*, vol. 22, pp. 465–75.

BURGER, J, KIRCHNER, M, BRAMANTI, B, HAAK, W, THOMAS, MG (2007), 'Absence of the lactase-persistence associated allele in early neolithic Europeans', *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, pp. 3736–41.

BURGESS, JW, NEVILLE, TA, ROUILLARD, P, HARDER, Z, BEANLANDS, DS, SPARKS, DL (2005), 'Phosphatidylinositol increases HDL-C levels in humans', *Journal of Lipid Research*, vol. 46, pp. 350–5.

CARRERO, JJ, FONOLLÁ, J, MARTI, JL, JIMÉNEZ, J, BOZA, JJ, LÓPEZ-HUERTAS, E (2007), 'Intake of fish oil, oleic acid, folic acid, and vitamins B-6 and E for 1 year decreases plasma C-reactive protein and reduces coronary heart disease risk factors in male patients in a cardiac rehabilitation program', *Journal of Nutrition*, vol. 137, pp. 384–90.

CARRUTH, BR, SKINNER, JD (2001), 'The role of dietary calcium and other nutrients in moderating body fat in preschool children', *International Journal of Obesity and Related Metabolic Disorders*, vol. 25, pp. 559–66.

CASHMAN, KD (2007), 'Diet, nutrition, and bone health', *Journal of Nutrition*, vol. 137, pp. 2507S–2512S.

CHERBUT, C (2002), 'Inulin and oligofructose in the dietary fiber concept', The *British Journal of Nutrition*, vol. 87, pp. S159–S162.

CHERMESH, I, ELIAKIM, R (2006), 'Probiotics and the gastrointestinal tract: Where are we in 2005?', *World Journal of Gastroenterology*, vol. 12, pp. 853–7.

CHOI, HK, WILLETT, WC, STAMPFER, MJ, RIMM, E, HU, FB (2005), 'Dairy consumption and risk of type 2 diabetes mellitus in men: a prospective study', *Archives of Internal Medicine*, vol. 165, pp. 997–1003.

COHN, JS (1998), 'Postprandial lipemia: Emerging evidence for atherogenicity of remnant lipoproteins', *Canadian Journal of Cardiology*, vol. 14, pp. 18B–27B.

COLECCHIA, A, VESTITO, A, LA ROCCA, A, PASQUI, F, NIKIFORAKI, A, FESTI, D, SYMBIOTIC STUDY GROUP (2006), 'Effect of a symbiotic preparation on the clinical manifestations of irritable bowel syndrome, constipation-variant. Results of an open, uncontrolled multicenter study', *Minerva Gastroenterologica e Dietologica*, vol. 52, no. 4, pp. 349–58.

COUZY, F, KASTENMAYER, P, VIGO, M, CLOUGH, J, MUNOZ-BOX R, BARCLAY, DV (1995), 'Calcium bioavailability from a calcium- and sulfate-rich mineral water, compared with milk, in young adult women', *American Journal of Clinical Nutrition*, vol. 62, pp. 1239–44.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

CREMONINI, F, DI CARO, S, NISTA, EC, BARTOLOZZI, F, CAPELLI, G, GASBARRINI, G, GASBARRINI, A (2002), 'Meta-analysis: the effect of probiotic administration on antibiotic-associated diarrhea', *Alimentary Pharmacology & Therapeutics*, vol. 16, pp. 1461–7.

CUMMINGS, JH, MACFARLANE, G (2002), 'Gastrointestinal effects of prebiotics', *British Journal of Nutrition*, vol. 87, pp. S145–S151.

CUMMINGS, JH, CHRISTIE, S, COLE, TJ (2001), 'A study of fructo oligosaccharides in the prevention of travellers' diarrhoea', *Alimentary Pharmacology & Therapeutics*, vol. 15, pp. 1139–45.

DAVIES, KM, HEANEY, RP, RECKER, RR, LAPPE, JM, BARGER-LUX, MJ, RAFFERTY, K, HINDERS, S (2000), 'Calcium intake and body weight', *Journal of Clinical Endocrinology and Metabolism*, vol. 8, pp. 4635–8.

DAWSON-HUGHES, B, BISCHOFF-FERRARI, HA (2007), 'Therapy of osteoporosis with calcium and vitamin D', *Journal of Bone and Mineral Research*, vol. 22, pp. V59–V63.

DE ROOS, NM, KATAN, M (2000), 'Effects of probiotic bacteria on diarrhea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998', *American Journal of Clinical Nutrition*, vol. 71, pp. 405–11.

DE VRESE, M (2003), 'Effects of probiotic bacteria on gastrointestinal symptoms, *Helicobacter pylori* activity and antibiotics-induced diarrhoea', *Gastroenterology*, vol. 124, p. A560.

DE VRESE, M, MARTEAU, PR (2007), 'Probiotics and prebiotics: effects on diarrhea', *Journal of Nutrition*, vol. 137, pp. 803S–811S.

DE VRESE, M, SCHREZENMEIR, J (2008), 'Probiotics, prebiotics, and synbiotics', *Advances in Biochemical Engineering/Biotechnology*, vol. 111, pp. 1–66.

DE VRESE, M, KUHN, C, TITZE, A, LORENZ, A, SUHR, M, BARTH, CA, SCHREZENMEIR, J (1997), 'Einfluß von Art und Viabilität von Laktobazillen in Sauermilchprodukten auf die Laktoseverdauung', *Aktuelle Ernährungsmedizin*, vol. 2, p. 44.

DE VRESE, M, STEGELMANN, A, RICHTER, B, FENSELAU, S, LAUE, C, SCHREZENMEIR, J (2001), 'Probiotics – compensation for lactase insufficiency', *American Journal of Clinical Nutrition*, vol. 73, pp. 361–4.

DENDUKURI, N, COSTA, V, MCGREGOR, M, BROPHY, JM (2005), 'Probiotic therapy for the prevention and treatment of *Clostridium difficile*-associated diarrhea: a systematic review', *Canadian Medical Association Journal*, vol. 173, pp. 167–70.

DENKE, MA, FOX, MM, SCHULTE, MC (1993), 'Short-term dietary calcium fortification increases fecal saturated fat content and reduces serum lipids in men', *Journal of Nutrition*, vol. 123, pp. 1047–53.

DEPARTMENT OF HEALTH (1998), 'Nutrition and Bone Health with Reference to Calcium and Vitamin D', Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. *Report on Health and Social Subjects*, no. 49. London: HM Stationery Office.

DEUTSCHE GESELLSCHAFT FÜR ERNÄHRUNG, ÖSTERREICHISCHE GESELLSCHAFT FÜR ERNÄHRUNG, SCHWEIZERISCHE VEREINIGUNG FÜR ERNÄHRUNG, SCHWEIZERISCHE GESELLSCHAFT FÜR ERNÄHRUNGSFORSCHUNG (2000), 'Referenzwerte für die Nährstoffzufuhr', Umschau Braus Verlag, Frankfurt.

DIETARY REFERENCE INTAKES FOR CALCIUM, PHOSPHORUS, MAGNESIUM, VITAMIN D, AND FLUORIDE (1997), National Academy of Sciences. Institute of Medicine. Food and Nutrition Board, Washington, DC.

DIETARY REFERENCE INTAKES FOR VITAMIN A, VITAMIN K, ARSENIC, BORON, CHROMIUM, COPPER, IODINE, IRON, MANGANESE, MOLYBDENUM, NICKEL, SILICON, VANADIUM, AND

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

ZINC (2001), National Academy of Sciences. Institute of Medicine. Food and Nutrition Board, Washington, DC.

DIETARY REFERENCE INTAKES FOR WATER, POTASSIUM, SODIUM, CHLORIDE, AND SULFATE (2005, National Academy of Sciences. Institute of Medicine. Food and Nutrition Board, Washington, DC.

DONTAS, IA, YIANNAKOPOULOS, CK (2007), 'Risk factors and prevention of osteoporosis-related fractures', *Journal of Musculoskeletal & Neuronal Interactions*, vol. 7, no. 3, pp. 268–72.

DOTAN, I, RACHMILEWITZ, D (2005), 'Probiotics in inflammatory bowel disease: possible mechanisms of action', *Current Opinion in Gastroenterology*, vol. 21, pp. 426–30.

ELWOOD, PC, PICKERING, JE, HUGHES, J, *ET AL.* (2004), 'Milk drinking, ischaemic heart disease and ischaemic stroke II. Evidence from cohort studies', *European Journal of Clinical Nutrition*, vol. 58, pp. 718–24.

FANARO, S, BOEHM, G, GARSSEN, J, KNOL, J, MOSCA, F, STAHL, B, VIGI, V (2005), 'Galacto-oligosaccharides and long-chain fructo-oligosaccharides as prebiotics in infant formulas: a review', *Acta Paediatrica*, Suppl, vol. 94, pp. 22–6.

FANIGLIULO, L, COMPARATO, G, ARAGONA, G, CAVALLAIO, L, IORI, V, MAINO, M, CAVESTRO, GM, SOLIANI, P, SIAMESI, M, FRANZE, A, DI MARIO, F (2006), 'Role of gut microflora and probiotic effects in the irritable bowel syndrome', *Acta Bio-medica*, vol. 77, pp. 85–9.

FAO/WHO (2001), 'Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria', *Report of a Joint FAO/WHO Expert Consultation 2001, Cordoba, Argentina*, vol. 1–4, pp. 19–20.

FARNWORTH, ER (ED.) (2008), *Handbook of Fermented Functional Food*, CRC Press, Boca Raton, FL.

FITZGERALD, RJ, MEISEL, H (2000), 'Milk protein-derived peptide inhibitors of angiotensin-I-converting enzyme', *British Journal of Nutrition*, vol. 84, pp. S33–S37.

FONDEN, R, MOGENSEN, G, TANAKA, R, SALMINEN, S (2000), 'Effect of fermented dairy products on intestinal microflora, human nutrition, and health: current knowledge and future perspectives', *Bulletin of the International Dairy Federation*, p. 352.

GERRIOR, S, BENTE, L (2002), 'Nutrient content of the US food supply, 1909–1999: A summary report', USDA Center for Nutrition Policy and Promotion, Washington, DC.

GERSTNER, G, DE VRESE, M (2007), 'Dietary and supplemental calcium and its role in weight loss: weighing the evidence', in: Henry, CJK (ed.), *Novel Food Ingredients for Weight Control*, Woodhead Publishing, Cambridge.

GIBSON, GR, ROBERFROID, MB (1995), 'Dietary modulation of the colonic microbiota: Introducing the concept of prebiotics', *Journal of Nutrition*, vol. 125, pp. 1401–12.

GILL, HS, SHU, Q, LIN, H, RUTHERFURD, KJ, CROSS, ML (2001), 'Protection against translocating *Salmonella typhimurium* infection in mice by feeding the immuno-enhancing probiotic *Lactobacillus rhamnosus* strain HN001', *Medical Microbiology and Immunology* (Berlin), vol. 190, pp. 97–104.

GIONCHETTI, P, RIZZELLO, F, LAMMERS, KM, MORSELLI, C, SOLLAZZI, L, DAVIES, S, TAMBASCO, R, CALABRESE, C, CAMPIERI, M (2006), 'Antibiotics and probiotics in treatment of inflammatory bowel disease', *World Journal of Gastroenterology*, vol. 12, pp. 3306–13.

GORI, AM, CORSI, AM, FEDI, S, GAZZINI, A, SOFI, F, BARTALI, B, BANDINELLI, S, GENSINI, GF, ABBATE, R, FERRUCCI, L (2005), 'A proinflammatory state is associated with hyperhomocysteinemia in the elderly', *American Journal of Clinical Nutrition*, vol. 82, pp. 335–41.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

GRUNDY, SM, CLEEMAN, JI, DANIELS, SR, *ET AL.* (2005), 'Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement', *Circulation*, vol. 112, pp. 2735–52.

HAN, JR, DENG, B, SUN, J, CHEN, CG, CORKEY, BE, KIRKLAND, JL, MA, J, GUO, W (2007), 'Effects of dietary medium-chain triglyceride on weight loss and insulin sensitivity in a group of moderately overweight free-living type 2 diabetic Chinese subjects', *Metabolism*, vol. 56, pp. 985–91.

HATAKKA, K, SAVILAHTI, E, PONKA, A, MEURMAN, JH, POUSSA, T, NASE, L, SAXELIN, M, KORPELA, R (2001), 'Effect of long term consumption of probiotic milk on infections in children attending day care centres: a double blind, randomised trial', *BMJ (Clinical Research edn)*, vol. 322, p. 1327.

HÄUSSLER, B, GOTHE, H, GÖL, D, GLAESKE, G, PIENTKA, L, FELSENBERG, D (2007), 'Epidemiology, treatment and costs of osteoporosis in Germany – the BoneEVA Study', *Osteoporosis International*, vol. 18, no. 1, pp. 77–84.

HAYATSU, H, HAYATSU, T (1993), 'Suppressing effect of *Lactobacillus casei* administration on the urinary mutagenicity arising from ingestion of fried ground beef in the human', *Cancer Letters*, vol. 73, pp. 173–9.

HEANEY, RP (2003), 'Normalizing calcium intake: projected population effects for body weight', *Journal of Nutrition*, vol. 133, pp. S268–S270.

HEANEY, RP (2004), 'Phosphorus nutrition and the treatment of osteoporosis', *Mayo Clinic Proceedings*, vol. 79, pp. 91–7.

HEANEY, RP (2007), 'Bone health', *American Journal of Clinical Nutrition*, vol. 85, pp. 300S–303S.

HEANEY, RP, WEAVER, CM (2005), 'Newer perspectives on calcium nutrition and bone quality', *Journal of the American College of Nutrition*, vol. 24, pp. 574S–581S.

HEANEY, RP, RECKER, RR, WEAVER, CM (1990), 'Absorbability of calcium sources: the limited role of solubility', *Calcified Tissue International*, vol. 46, pp. 300–4.

HEANEY, RP, ABRAMS, S, DAWSON-HUGHES, B, LOOKER, A, MARCUS, R, MATKOVIC, V, WEAVER, C (2000), 'Peak bone mass', *Osteoporosis International*, vol. 11, pp. 985–1009.

HEANEY, RP, DAVIES, KM, BARGER-LUX, MJ (2002), 'Calcium and weight: clinical studies', *Journal of the American College of Nutrition*, vol. 21, pp. S152–S155.

HERRERA, JA, AREVALO-HERRERA, M, SHAHABUDDIN, AK, ERSHENG, G, HERRERA, S, GARCIA, RG, LOPEZ-JARAMILLO, P (2006), 'Calcium and conjugated linoleic acid reduces pregnancy-induced hypertension and decreases intracellular calcium in lymphocytes', *American Journal of Hypertension*, vol. 19, pp. 381–7.

HILTON, E, KOLAKOWSKI, P, SMITH, M, SINGER, C (1997), 'Efficacy of *Lactobacillus* GG as a diarrhoea preventive', *Journal of Travel Medicine*, vol. 4, pp. 3–7.

HUANG, JS, BOUSVAROS, A, LEE, JW, DIAZ, A, DAVIDSON, EJ (2002), 'Efficacy of probiotic use in acute diarrhea in children: a meta-analysis', *Digestive Diseases and Sciences*, vol. 47, pp. 2625–34.

HUTH, PJ, DIRIENZO, DB, MILLER, GD (2006), 'Major scientific advances with dairy foods in nutrition and health', *Journal of Dairy Science*, vol. 89, pp. 1207–21.

INNIS, SM, DAVIDSON, AG, MELYNK, S, JAMES, SJ (2007), 'Choline-related supplements improve abnormal plasma methionine–homocysteine metabolites and glutathione status in children with cystic fibrosis', *American Journal of Clinical Nutrition*, vol. 85, pp. 702–8.

INOUE, N, NAGAO, K, HIRATA, J, WANG, YM, YANAGITA, T (2004), 'Conjugated linoleic acid prevents the development of essential hypertension in spontaneously hypertensive rats', *Biochemical and Biophysical Research Communications*, vol. 323, pp. 679–84.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

ISHIKAWA, H, AKEDO, I, UMESAKI, Y, TANAKA, R, IMAOKA, A, OTANI, T (2003), 'Randomized controlled trial on the effect of bifidobacteria-fermented milk on ulcerative colitis', *Journal of the American College of Nutrition*, vol. 22, pp. 56–63.

JACQMAIN, M, DOUCET, E, DESPRES, J-P, BOUCHARD, C (2003), 'Calcium intake, body composition, and lipoprotein-lipid concentrations in adults', *American Journal of Clinical Nutrition*, vol. 77, pp. 1448–52.

JAUHIAINEN, T, KORPELA, R (2007), 'Milk peptides and blood pressure', *Journal of Nutrition*, vol. 137, pp. 825S–829S.

KAJANDER, K, KORPELA, R (2006), 'Clinical studies on alleviating the symptoms of irritable bowel syndrome', *Asia Pacific Journal of Clinical Nutrition*, vol. 15, no. 4, pp. 576–80.

KANIS, JA, JOHNELL, O (2005), 'Requirements for DXA for the management of osteoporosis in Europe', *Osteoporosis International*, vol. 16, p. 229.

KASHKET, S, DEPAOLA, DP (2002), 'Cheese consumption and the development and progression of dental caries', *Nutrition Reviews*, vol. 60, pp. 97–103.

KATELARIS, PH, SALAM, I, FARTHING, MJG (1995), 'Lactobacilli to prevent traveler's diarrhea?', *New England Journal of Medicine*, vol. 33, pp. 1360–1.

KERSTETTER, JE, ALLEN, LH (1994), 'Protein intake and calcium homeostasis', *Advances in Nutritional Research*, vol. 9, pp. 167–81.

KLEIN-PLATAT, C, DRAI, J, OUJAA, M, SCHLIENGER, JL, SIMON, C (2005), 'Plasma fatty acid composition is associated with the metabolic syndrome and low-grade inflammation in overweight adolescents', *American Journal of Clinical Nutrition*, vol. 82, pp. 1178–84.

KNOL, J, BOEHM, G, LIDESTRI, M, NEGRETTI, F, JELINEK, J, AGOSTI, M, STAHL, B, MARINI, A, MOSCA, F (2005), 'Increase of faecal bifidobacteria due to dietary oligosaccharides induces a reduction of clinically relevant pathogen germs in the faeces of formula-fed preterm infants', *Acta Paediatrica*, vol. 94, Suppl, no. 449, pp. 31–3.

KOEBNICK, C, WAGNER, I, LEITZMANN, P, STERN, U, ZUNFT, HJF (2003), 'Probiotic beverage containing *Lactobacillus casei* Shirota improves gastrointestinal symptoms in patients with chronic constipation', *Canadian Journal of Gastroenterology*, vol. 17, pp. 655–9.

KOSHIHARA, M, MASUYAMA, R, UEHARA, M, SUZUKI, K (2004), 'Effect of dietary calcium: Phosphorus ratio on bone mineralization and intestinal calcium absorption in ovariectomized rats', *Biofactors*, vol. 22, no. 1–4, pp. 39–42.

KRAMMER, HJ, KAMPER, H, VON BUNAU, R, ZIESENISS, E, STANGE, C, SCHLIEGER, F, CLEVER, I, SCHULZE, J (2006), 'Probiotic drug therapy with *E. coli* strain Nissle 1917 (EcN): Results of a prospective study of the records of 3807 patients', *Zeitschrift für Gastroenterologie*, vol. 44, pp. 651–6.

LANOU, AJ, BERKOW, SE, BARNARD, ND (2005), 'Calcium, dairy products, and bone health in children and young adults: a reevaluation of the evidence', *Pediatrics*, vol. 115, no. 3, pp. 736–43.

LARSEN, TM, TOUBRO, S, ASTRUP, A (2003), 'Efficacy and safety of dietary supplements containing CLA for the treatment of obesity: evidence from animal and human studies', *Journal of Lipid Research*, vol. 44, pp. 2234–41.

LEWIS, S, BURMEISTER, S, BRAZIER, J (2005), 'Effect of the prebiotic oligofructose on relapse of *Clostridium difficile*-associated diarrhea: a randomized, controlled study', *Clinical Gastroenterology and Hepatology*, vol. 3, pp. 442–8.

LICHTENSTEIN, AH, ERKKILÄ, AT, LAMARCHE, B, SCHWAB, US, JALBERT, SM, AUSMAN, LM (2003), 'Influence of hydrogenated fat and butter on CVD risk factors: Remnant-

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

like particles, glucose and insulin, blood pressure and C-reactive protein', A*therosclerosis*, vol. 171, pp. 97–107.

LIN, YC, LYLE, RM, MCCABE, LD, MCCABE, GP, WEAVER, CM, TEEGARDEN, D (2000), 'Dairy calcium is related to changes in body composition during a two-year exercise intervention in young women', *Journal of the American College of Nutrition*, vol. 19, no. 6, pp. 754–60.

LINDSAY, JO, WHELAN, K, STAGG, AJ, GOBIN, P, AL-HASSI, HO, RAYMENT, N, KAMM, MA, KNIGHT, SC, FORBES, A (2006), 'Clinical, microbiological, and immunological effects of fructo-oligosaccharide in patients with Crohn's disease', *Gut*, vol. 55, pp. 348–55.

LOOS, RJ, RANKINEN, T, LEON, AS, SKINNER, JS, WILMORE, JH, RAO, DC, BOUCHARD, C (2004), 'Calcium intake is associated with adiposity in Black and White men and White women of the HERITAGE Family Study', *Journal of Nutrition*, vol. 134, pp. 1772–8.

LUHOVYY, BL, AKHAVAN, T, ANDERSON, GH (2007), 'Whey proteins in the regulation of food intake and satiety', *Journal of the American College of Nutrition*, vol. 26, no. 6, pp. 704S–712S.

LUTSEY, PL, STEFFEN, LM, FELDMAN, HA, HOELSCHER, DH, WEBBER, LS, LUEPKER, RV, LYTLE, LA, ZIVE, M, OSGANIAN, SK (2006), 'Serum homocysteine is related to food intake in adolescents: the Child and Adolescent Trial for Cardiovascular Health', *American Journal of Clinical Nutrition*, vol. 83, pp. 1380–6.

MAJAMAA, H, ISOLAURI, E, SAXELIN, M, VESIKARI, T (1995), 'Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis', *Journal of Pediatric Gastroenterology and Nutrition*, vol. 20, pp. 333–8.

MALPUECH-BRUGÈRE, C, VERBOEKET-VAN DE VENNE, WPHG, MENSINK, RP, ARNAL, M-A, MORIO, B, BRANDOLINI, M (2004), 'Effects of two conjugated linoleic acid isomers on bodyfat mass in overweight humans', *Obesity Research*, vol. 12, pp. 591–8.

MARTEAU, P, DE VRESE, M, CELLIER, C, SCHREZENMEIR, J (2001), 'Protection from gastrointestinal diseases using probiotics', *American Journal of Clinical Nutrition*, vol. 73, pp. 430S–436S.

MARTEAU, P, LEMANN, M, SEKSIK, P, *ET AL.* (2006), 'Ineffectiveness of *Lactobacillus johnsonii* LA1 for prophylaxis of postoperative recurrence in Crohn's disease: a randomised, double-blind, placebo-controlled GETAID trial', *Gut*, vol. 55, pp. 842–7.

MARTEN, B, PFEUFFER, M, SCHREZENMEIR, J (2006), 'Medium chain triglycerides', *International Dairy Journal*, vol. 16, pp. 1374–82.

MASSEY, L (2001), 'Dairy food consumption, blood pressure and stroke', *Journal of Nutrition*, vol. 131, pp. 1875–8.

MASUYAMA, R, NAKAYA Y, KATSUMATA S (2003), 'Dietary calcium and phosphorus ratio regulates bone mineralization and turnover in vitamin D receptor knockout mice by affecting intestinal calcium and phosphorus absorption', *Journal of Bone and Mineral Research*, vol. 18, no. 7, pp. 1217–26.

MATSUI, T, KAWAKITA, Y, YANO, H (1997), 'Dietary skim milk powder increase ionized calcium in the small intestine of piglets compared to dietary defatted soybean flour', *Journal of Nutrition*, vol. 127, pp. 1357–61.

MCFARLAND, LV (2006), 'Meta-analysis of probiotics for the prevention of antibiotic-associated diarrhea and the treatment of *Clostridium difficile* disease', *American Journal of Gastroenterology*, vol. 101, pp. 812–22.

MELA, DJ (ED.) (2005), *Food, Diet and Obesity*, Woodhead Publishing, Cambridge.

MICKE, P, BEEH, KM, BUHL, R (2002), 'Effects of long-term supplementation with whey

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

proteins on plasma glutathione levels of HIV-infected patients', *European Journal of Nutrition*, vol. 41, pp. 12–18.

MITSUOKA, T (1992), 'Intestinal flora and ageing', *Nutrition Reviews*, vol. 50, pp. 438–46.

MÖLLER, NP, SCHOLZ-AHRENS, KE, ROOS, N, SCHREZENMEIR, J (2008), 'Bioactive peptides and proteins from foods: indication for health effects', *European Journal of Nutrition*, vol. 47, pp. 171–82.

NAGAO, K, INOUE, N, WANG, YM, YANAGITA, T (2003), 'Conjugated linoleic acid enhanced plasma adiponektin level and alleviates hyperinsulinemia and hypertension in Zucker diabetic fatty (fa/fa) rats', *Biochemical and Biophysical Research Communications*, vol. 310, pp. 562–6.

NATIONAL INSTITUTES OF HEALTH (2001), 'Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)', Bethesda, MD: National Institutes of Health, NIH Publication 01-3670.

NAVAB, M, HAMA, S, HOUGH, G, FOGELMAN, AM (2003), 'Oral synthetic phospholipid (DMPC) raises high-density lipoprotein cholesterol levels, improves high-density lipoprotein function, and markedly reduces atherosclerosis in apolipoprotein E-null mice', *Circulation*, vol. 108, pp. 1735–9.

NAZARE, JA, DE LA PERRIÈRE, AB, BONNET, F, *ET AL.* (2007), 'Daily intake of conjugated linoleic acid-enriched yoghurts: effects on energy metabolism and adipose tissue gene expression in healthy subjects', *British Journal of Nutrition*, vol. 97, no. 2, pp. 273–80.

NICKEL, KP, MARTIN, BR, SMITH, DL, SMITH, JB, MILLER, GD, WEAVER, CM (1996), 'Calcium bioavailability from bovine milk and dairy products in premenopausal women using intrinsic and extrinsic labeling techniques', *Journal of Nutrition*, vol. 126, no. 5, pp. 1406–11.

NOH, SK, KOO, SI (2004), 'Milk sphingomyelin is more effective than egg sphingomyelin in inhibiting intestinal absorption of cholesterol and fat in rats', *Journal of Nutrition*, vol. 134, pp. 2611–16.

OBARZANEK, E, SACKS, FM, VOLLMER, WM, PROSCHAN, MA, DASH RESEARCH GROUP, *ET AL.* (2001), 'Effects on blood lipids of a blood pressure-lowering diet: the Dietary Approaches to Stop Hypertension (DASH) trial', *American Journal of Clinical Nutrition*, vol. 74, pp. 80–9.

OBERMAYER-PIETSCH, BM, GUGATSCHKA, M, REITTER, S, *ET AL.* (2007), 'Adult-type hypolactasia and calcium availability: decreased calcium intake or impaired calcium absorption?', *Osteoporosis International*, vol. 18, pp. 445–51.

OLTHOF, MR, BRINK, EJ, KATAN, MB, VERHOEF, P (2005), 'Choline supplemented as phosphatidylcholine decreases fasting and postmethionine-loading plasma homocysteine concentrations in healthy men', *American Journal of Clinical Nutrition*, vol. 82, pp. 111–17.

OUWEHAND, AC, LAGSTRÖM, H, SUOMALAINEN, T, SALMINEN, S (2002), 'The effect of probiotics on constipation, faecal azoreductase activity and facal mucins', *Annals of Nutrition & Metabolism*, vol. 10, pp. 159–62.

PEDONE, CA, BERNABEU, AO, POSTAIRE, ER, BOULEY, CF, REINERT, P (1999), 'The effect of supplementation with milk fermented by *Lactobacillus casei* DN-114 001, on acute diarrhoea in children attending day care centres', *International Journal of Clinical Practice*, vol. 53, pp. 179–84.

PEDONE, CA, ARNAUD, CC, POSTAIRE, ER, BOULEY, CF, REINERT, P (2000), 'Multicenter study on 928 children attending day care centers to evaluate the effect of milk fermented

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

by *Lactobacillus casei* DN-114 001 on the incidence of diarrhoea', *International Journal of Clinical Practice*, vol. 54, pp. 568–71.

PEREIRA, MA, JACOBS, DR JR, VAN HORN, L, SLATTERY, ML, KARTASHOV, AI, LUDWIG, DS (2002), 'Dairy consumption, obesity, and the insulin resistance syndrome in young adults: the CARDIA study', *JAMA*, vol. 287, pp. 2081–9.

PFEUFFER, M, SCHREZENMEIR, J (2007), 'Milk and the metabolic syndrome', *Obesity Reviews*, vol. 8, pp. 109–18.

PING-DELFOS, WC, SOARES, MJ, CUMMINGS, NK (2004), 'Acute suppression of spontaneous food intake following dairy calcium and vitamin D', *Asia Pacific Journal of Clinical Nutrition*, vol. 13, p. S82.

POPPITT, SD, KEOGH, GF, MULVEY, TB, MCARDLE, BH, MACGIBBON, AKH, COOPER, GJS (2002), 'Lipid-lowering effects of a modified butter-fat: a controlled intervention trial in healthy men', *European Journal of Clinical Nutrition*, vol. 56, pp. 64–71.

PRENTICE, A, SCHOENMAKERS, I, LASKEY, MA, DE BONO, S, GINTY, F, GOLDBERG, GR (2006), 'Nutrition and bone growth and development', *Proceedings of the Nutrition Society*, vol. 65, pp. 348–60.

PRZYREMBEL, H (2005), 'EFSA Risikobewertung von Vitaminen und Mineralstoffen', BLL-Conference 'Food Supplements in Europe', 20 September 2005, Berlin.

RAFTER, JJ (1995), 'The role of lactic acid bacteria in colon cancer prevention', *Scandinavian Journal of Gastroenterology*, vol. 30, pp. 497–502.

RAFTER, J, BENNETT, M, CADERNI, G, *ET AL.* (2007), 'Dietary synbiotics reduce cancer risk factors in polypectomized and colon cancer patients', *American Journal of Clinical Nutrition*, vol. 85, no. 2, pp. 488–96.

REFERENZWERTE FÜR DIE NÄHRSTOFFZUFUHR DER DEUTSCHEN, ÖSTERREICHISCHEN UND SCHWEIZERISCHEN GESELLSCHAFTEN FÜR ERNÄHRUNG (D-A-CH) (2000), Umschau Braus Verlag, Frankfurt, pp. 159–168.

ROBERFROID, M (2007), 'Prebiotics: the concept revisited', *Journal of Nutrition*, vol. 137, pp. 830S–837S.

RODRIGO, R, PRAT, H, PASSALACQUA, W, ARAYA, J, GUICHARD, C, BÄCHLER, JP (2007), 'Relationship between oxidative stress and essential hypertension', *Hypertension Research*, vol. 30, pp. 1159–67.

ROLLAND, V, ROSEAU, S, FROMENTIN, G, NICOLAIDIS, S, TOMÉ, D, EVEN, PC (2002), 'Body weight, body composition, and energy metabolism in lean and obese Zucker rats fed soybean oil and butter', *American Journal of Clinical Nutrition*, vol. 75, pp. 21–30.

ROSS, R (1993), 'The pathogenesis of atherosclerosis: a perspective for the 1990s', *Nature*, vol. 362, pp. 801–9.

ROUX, C, BISCHOFF-FERRARI, HA, PAPAPOULOS, SE, DE PAPP, AE, WEST, JA, BOUILLON, R (2008), 'New insights into the role of vitamin D and calcium in osteoporosis management: an expert roundtable discussion', *Current Medical Research and Opinion*, vol. 24, pp. 1363–70.

SACKS, FM, SVETKEY, LP, VOLLMER, WM, *ET AL.*, DASH – SODIUM COLLABORATIVE RESEARCH GROUP (2001), 'Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet', *New England Journal of Medicine*, vol. 344, pp. 3–10.

SAMUELSON, G, BRATTEBY, LE, MOHSEN, R, VESSBY, B (2001), 'Dietary intake in healthy adolescents: inverse relationship between the estimated intake of saturated fatty acids and serum cholesterol', *British Journal of Nutrition*, vol. 85, pp. 333–41.

SARZANI, R, SALVI, F, DESSÌ-FULGHERI, P, RAPPELLI, A (2008), 'Renin–angiotensin system,

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

natriuretic peptides, obesity, metabolic syndrome, and hypertension: an integrated view in humans', *Journal of Hypertension*, vol. 26, no. 5, pp. 831–43.

SAZAWAL, S, HIREMATH, G, DHINGRA, U, MALIK, P, DEB, S, BLACK, RE (2006), 'Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials', *Lancet Infectious Diseases*, vol. 6, pp. 374–82.

SCHOLZ-AHRENS, KE, SCHREZENMEIR, J (2000), 'Effects of bioactive substances in milk on mineral and trace element metabolism with special reference to casein phosphopeptides', *British Journal of Nutrition*, vol. 84, Suppl 1, pp. S147–S153.

SCHOLZ-AHRENS, KE, SCHREZENMEIR, J (2006), 'Milk minerals and the metabolic syndrome', *International Dairy Journal*, vol. 16, pp. 1399–407.

SCHOLZ-AHRENS, KE, SCHREZENMEIR, J (2007), 'Inulin and oligofructose and mineral metabolism: the evidence from animal trials', *Journal of Nutrition*, vol. 137, pp. 2513S–2523S.

SCHOLZ-AHRENS, K, SCHAAFSMA, G, VAN DEN HEUVEL, EG, SCHREZENMEIR, J (2001), 'Effects of prebiotics on mineral metabolism', *American Journal of Clinical Nutrition*, vol. 73, pp. 459S–464S.

SCHOLZ-AHRENS, KE, AÇIL, Y, SCHREZENMEIR, J (2002), 'Effect of oligofructose or dietary calcium on repeated calcium and phosphorus balances, bone mineralization and trabecular structure in ovariectomized rats', *British Journal of Nutrition*, vol. 88, pp. 365–77.

SCHOLZ-AHRENS, KE, ADE, P, MARTEN, B, WEBER, P, TIMM, W, AÇIL, Y, GLÜER, CC, SCHREZENMEIR, J (2007), 'Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure', *Journal of Nutrition*, vol. 137, no. 3, Suppl 2, pp. 838S–846S.

SCRIMSHAW, NS, MURRAY, EB (1988), 'The acceptability of milk and milk products in populations with a high prevalence of lactose intolerance', *American Journal of Clinical Nutrition*, vol. 48, pp. 1079–1159.

SHAHKHALILI, Y, MURSET, C, MEIRIM, I, DURUZ, E, GUINCHARD, S, CAVADINI, C, ACHESON, K (2001), 'Calcium supplementation of chocolate: effect on cocoa butter digestibility and blood lipids in humans', *American Journal of Clinical Nutrition*, vol. 73, pp. 246–52.

SHANAHAN, F (2000), 'Probiotics and inflammatory bowel disease: is there a scientific rationale?', *Inflammatory Bowel Diseases*, vol. 6, pp. 107–15.

SHARMA, AM (2008), 'The value of current interventions for obesity', *Nature Clinical Practice. Cardiovascular Medicine*, vol. 5, Suppl 1, pp. S3–S9.

SHI, H, DIRIENZO, D, ZEMEL, MB (2001), 'Effects of dietary calcium on adipocyte lipid metabolism and body weight regulation in energy-restricted aP2-agouti transgenic mice', *FASEB Journal*, vol. 15, pp. 291–3.

SHI, H, NORMAN, AW, OKAMURA, WH, SEN, A, ZEMEL, MB (2002), '1alpha,25-dihydroxyvitamin D3 inhibits uncoupling protein 2 expression in human adipocytes', *FASEB Journal*, vol. 16, no. 1, pp. 1808–10.

SMEDMAN, EM, GUSTAFFSON, I-B, BERGLUND, LGT, VESSBY, BOH (1999), 'Pentadecenoic acid in serum as a marker for intake of milk fat: relations between intake of milk fat and metabolic risk factors', *American Journal of Clinical Nutrition*, vol. 69, pp. 22–9.

STEFFEN, LM, KROENKE, CH, YU, X, PEREIRA, MA, SLATTERY, ML, VAN HORN, L, GROSS, MD, JACOBS, DR JR (2005), 'Associations of plant food, dairy product, and meat intakes with 15-y incidence of elevated blood pressure in young black and white adults: the Coronary Artery Risk Development in Young Adults (CARDIA) study',

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

*American Journal of Clinical Nutrition*, vol. 82, pp. 1169–77.

STRAUB, DA (2007), 'Calcium supplementation in clinical practice: a review of forms, doses, and indications', *Nutrition in Clinical Practice*, vol. 22, no. 3, pp. 286–96.

SZAJEWSKA, H, RUSZCZYNSKI, M, RADZIKOWSKI, A (2006), 'Probiotics in the prevention of antibiotic-associated diarrhea in children: a meta-analysis of randomized controlled trials', *Journal of Pediatrics*, vol. 149, pp. 367–72.

TANG, BM, ESLICK, GD, NOWSON, C, SMITH, C, BENSOUSSAN, A (2007), 'Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis', *Lancet*, vol. 370, pp. 657–66.

TAVANI, A, GALLUS, S, NEGRI, E, LA VECCHIA, C (2002), 'Milk, dairy products, and coronary heart disease', *Journal of Epidemiology and Community Health*, vol. 56, no. 6, pp. 471–2.

TAYLOR, JSW, WILLIAMS, SRP, RHYS, R, JAMES, P, FRENNEAUX, MP (2006), 'Conjugated linoleic acid impairs endothelial function', *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 26, pp. 307–12.

THOM, E, WADSTEIN, J, GUDMUNDSEN, O (2001), 'Conjugated linoleic acid reduces body fat in healthy exercising humans', *Journal of International Medical Research*, vol. 29, pp. 392–6.

TSUJI, H, KASAI, M, TAKEUCHI, H, NAKAMURA, M, OKAZAKI, M, KONDO, K (2001), 'Dietary medium-chain triacylglycerols suppress accumulation of body fat in a double-blind controlled trial in healthy men and women', *Journal of Nutrition*, vol. 131, pp. 2853–9.

VASKONEN, T (2003), 'Dietary minerals and modification of cardiovascular risk factors', *Journal of Nutritional Biochemistry*, vol. 14, pp. 492–506.

WALKER, WA (2000), 'Role of nutrients and bacterial colonization in the development of intestinal host defense', *Journal of Pediatric Gastroenterology and Nutrition*, vol. 30, pp. S2–S7.

WARENSJÖ, E, JANSSON, JH, BERGLUND, L, *ET AL.* (2004), 'Estimated intake of milk fat is negatively associated with cardiovascular risk factors and does not increase the risk of a first acute myocardial infarction. A prospective case-control study', *British Journal of Nutrition*, vol. 91, pp. 635–42.

WATRAS, AC, BUCHHOLZ, AC, CLOSE, RN, ZHANG, Z, SCHOELLER, DA (2007), 'The role of conjugated linoleic acid in reducing body fat and preventing holiday weight gain', *International Journal of Obesity* (London), vol. 31, pp. 481–7.

WEAVER, CM (2008), 'The role of nutrition on optimizing peak bone mass', *Asia Pacific Journal of Clinical Nutrition*, vol. 17, pp. S135–S137.

WEAVER, CM, PROULX, WR, HEANEY, R (1999), 'Choices for achieving adequate dietary calcium with a vegetarian diet', *American Journal of Clinical Nutrition*, vol. 70, no. 3, Suppl, pp. 543S–548S.

WEIZMAN, Z, ASLI, G, ALSHEIKH, A (2005), 'Effect of a probiotic infant formula on infections in child care centers: comparison of two probiotic agents', *Pediatrics*, vol. 115, pp. 5–9.

WERNER, E, HANSEN, CH, ROTH, P, KALTWASSER, JP (1999), 'Intestinal absorption of calcium from foodstuffs as compared to a pharmaceutical preparation', *Isotopes in Environmental and Health Studies*, vol. 35, no. 1–2, pp. 111–18.

WHIGHAM, LD, O'SHEA, M, MOHEDE, IC, WALASKI, HP, ATKINSON, RL (2004), 'Safety profile of conjugated linoleic acid in a 12-month trial in obese humans', *Food and Chemical Toxicology*, vol. 42, no. 10, pp. 1701–9.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

WHIGHAM, LD, WATRAS, AC, SCHOELLER, DA (2007), 'Efficacy of conjugated linoleic acid for reducing fat mass: a meta-analysis in humans', *American Journal of Clinical Nutrition*, vol. 85, pp. 1203–11.

WHO (2000), 'Obesity: preventing and managing the global epidemic. Report of a WHO consultation', *WHO Technical Report Series*, vol. 894.

WINZENBERG, T, SHAW, K, FRYER, J, JONES G (2006), 'Effects of calcium supplementation on bone mineral density in healthy children: meta-analysis of randomised controlled trials', *BMJ (Clinical Research edn)*, vol. 333, pp. 775–81.

WOSJE, KS, KALKWARF, HJ (2004), 'Lactation, weaning, and calcium supplementation: effects on body composition in postpartum women', *American Journal of Clinical Nutrition*, vol. 80, pp. 423–9.

WOSJE, KS, SPECKER, BL (2000), 'Role of calcium in bone health during childhood', *Nutrition Reviews*, vol. 58, pp. 253–68.

XIAO, JZ, KONDO, S, TAKAHASHI, N, MIYAJI, K, OSHIDA, K, HIRAMATSU, A, IWATSUKI, K, KOKUBO, S, HOSONO, A (2003), 'Effects of milk products fermented by *Bifidobacterium longum* on blood lipids in rats and healthy adult male volunteers', *Journal of Dairy Science*, vol. 86, pp. 2452–61.

YADAV, H, JAIN, S, SINHA, PR (2007), 'Antidiabetic effect of probiotic dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* in high fructose fed rats', *Nutrition*, vol. 23, pp. 62–8.

ZAMBELL, KL, KEIM, NL, VAN LOAN, MD, GALE, B, BENITO, P, KELLEY, DS, NELSON, GJ (2000), 'Conjugated linoleic acid supplementation in humans: Effects on body composition and energy expenditure', *Lipids*, vol. 35, pp. 777–82.

ZEMEL, MB (2002), 'Regulation of adiposity and obesity risk by dietary calcium: Mechanisms and implications', *Journal of the American College of Nutrition*, vol. 21, pp. S146–S151.

ZEMEL, MB, ZEMEL, PC, BRYG, RJ, SOWERS, JR (1990), 'Dietary calcium induces regression of left ventricular hypertrophy in hypertensive non-insulin-dependent diabetic blacks', *American Journal of Hypertension*, vol. 3, pp. 458–63.

ZEMEL, MB, SHI, H, GREER, B, DIRIENZO, D, ZEMEL, PC (2000), 'Regulation of adiposity by dietary calcium', *FASEB Journal*, vol. 14, pp. 1132–8.

ZHONG, Y, PRIEBE, MG, VONK, RJ, HUANG, CY, ANTOINE, JM, HE, T, HARMSEN, HJ, WELLING, GW (2004), 'The role of colonic microbiota in lactose intolerance', *Digestive Diseases and Sciences*, vol. 49, pp. 78–83.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

# 3

# ‘Designer’ milks: functional foods from milk

**M. Boland, Riddet Institute, Massey University, New Zealand**

**Abstract:** Milk contains a range of bioactive components with potentially beneficial effects. Bioactive components of note include proteins such as lactoferrin and the immunoglobulins, peptides formed during milk protein hydrolysis, fatty acids such as conjugated linoleic acid, minerals, oligosaccharides and melatonin. Enhancement of bioactive components of milk is possible through a range of on-farm management processes, and also through processing to enrich the desired components. A range of milks with enhanced properties is commercially available, albeit with relatively slight supporting clinical evidence of effectiveness. With continuing elucidation of these beneficial effects and their mechanisms, demand will increase and, eventually, label claims are likely. There is increasing consumer awareness of the relationship between nutrition and health, with functional foods increasingly available as mainstream food lines in supermarkets. With the emergence of nutrigenomics, and awareness of personal nutritional needs, there will be increasing demand for functional milks and their products. Development of new, more sophisticated processing, including processes that are less likely to denature proteins, and processes to recover enriched streams of specific bioactives, will lead to wider availability of milk-derived functional foods and ingredients.

**Key words:** functional foods, cows’ milk, milk proteins, milkfat, lactoferrin, IgG, CLA, melatonin.

IP Address: 129.132.208.100

## 3.1 Introduction: functional milk components

Over the past decade and more, it has become clear that the role of food we consume can be much more than just nutrition. This has resulted in the emergence

© Woodhead Publishing Limited, 2010

Downloaded from Vet-eBooks.com

of functional foods: foods that confer health benefits over and above their nutritional value. Functional food has been defined by Diplock *et al.* (1999):

> 'A food can be regarded as functional if it has beneficial effects on target functions in the body beyond nutritional effects in a way that is relevant to health and well-being and/or the reduction of disease'.

Milk contains a wide range of components that have had beneficial health properties attributed to them. Some milks are considered to have improved health benefits through changes ('natural' or otherwise) in their composition. This creates the opportunity for specialised 'designer' milks that are either functional foods in their own right, or enriched sources for functional dairy ingredients.

The normal composition of milk is dealt with elsewhere in this volume and will not be repeated here. This chapter will first review components in milk with functional properties, and then discuss various means by which these may be altered in the production and processing of milk. A brief discussion of efficacy will follow, where reliable information is available.

Claims of functional effects of different foods abound in the literature, and results of new studies are appearing on a weekly basis. Claims based on a single isolated study should generally be regarded as unproven. A sound proof of functional efficacy will need to have most of the following elements:

- Proof of efficacy based on strictly controlled animal studies, using an appropriate animal model
- Identification of the probable biochemical mechanism, usually as a result of detailed *in vitro* and/or animal investigation
- Validation in at least one human clinical trial, of appropriate design, with a statistically robust outcome
- In most cases, it is preferred to have robust metadata, based on several independent studies, before functional properties can be regarded as really proven.

IP Address: 129.132.208.100

Many claims of functional effects come initially from epidemiological 'ecological' studies. Such studies are paper exercises that compare statistical data for health and mortality between different groups (usually different countries or regions) with consumption of various food types. While such studies can highlight associations, they have no value in proving cause and effect and must be treated as no more than an indication of the need for further investigation. Other studies use animal models only, often rodents, and these may not translate well to humans. This chapter is focused on health benefits that have been well substantiated, but refers to others where they may be important because of commercial exploitation and/or raised consumer expectations.

### 3.1.1 Protein and peptide components

Milk contains a range of whey proteins that have actual or potential bioactive applications. In addition, there is a range of bioactive peptides that are formed when milk proteins are digested or treated with enzymes in an industrial process.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

*Lactoferrin*

Lactoferrin is a minor whey protein in bovine milk, but is present at much higher levels in human milk, and so is of special interest for use in infant formulas. It is considered important because of the antibacterial properties of lactoferrin itself (reviewed by Orsi, 2004), usually attributed to its ability to scavenge iron, thus making it unavailable for bacterial growth, and of its breakdown peptide lactoferricin, which has very specific antibacterial properties and is believed to bind specifically to bacterial cell wall components (Orsi, 2004). Antibacterial effects have been demonstrated in clinical trials for improvement of colonic flora, treatment for *Helicobacter pylori*, and amelioration of hepatitis C (Wakabayashi *et al.*, 2006). In addition to its antimicrobial properties, lactoferrin has importance as an iron carrier, and it binds to some types of immune cells, which may have antiviral, antiinflammatory and immunomodulatory consequences. Lactoferrin has recently been implicated as a potent bone growth factor (Naot *et al.*, 2005). Many of these actions, other than anti-infection effects, have been demonstrated only in *in vitro* or *ex vivo* systems, and the actual role as a nutraceutical (i.e. an effect in humans from dietary intake) has yet to be demonstrated.

*Lactoperoxidase*

Along with lactoferrin, lactoperoxidase is thought to assist with inhibition of microbial growth (Boots and Floris, 2006). Lactoperoxidase is usually a sought-after ingredient for its value in a lactoperoxidase-based food preservation system (e.g. Touch *et al.*, 2004) rather than a specific bioactive; however, its presence in tears and saliva points to an important biological role, and future possible use in functional foods should not be discounted.

IP Address: 129.132.208.100

*Immunoglobulins*

Immunoglobulins in cows' milk have the potential to confer passive immunity in the gut. Cows' milk contains appreciable levels of IgG and rather low levels of IgM and IgA (Table 3.1). Colostrum has much higher levels of immunoglobulins. Stimulation of immunoglobulin production, particularly for Igs with activity against gut pathogens, has long been seen as desirable, conferring passive immunity to undesirable gut bacteria. Both colostrum and milks with enhanced levels of Igs have been produced and sold commercially for many years, for both human and animal consumption.

*β-Lactoglobulin*

The major whey protein, β-lactoglobulin, is widely regarded as the main cause of allergic reaction to milk (see, for example, Heinzmann *et al.*, 1999) and, in contrast to the other proteins listed here, is often regarded as a protein to be reduced or eliminated from milk. Human milk does not contain detectable levels of β-lactoglobulin, and nor does that of a range of non-ungulate mammals. For this reason, there has been ongoing interest in finding milk with reduced levels of this protein. The biological function of β-lactoglobulin in milk has never been

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

**Table 3.1** Levels of immunoglobulins and growth factors in milk and colostrum

| Immunoglobulin class | Level in milk (g/L) | Level in colostrum (g/L) |
|---|---|---|
| IgG | 0.47 | 60 |
| IgA | 0.05–0.1 | 3.5 |
| IgM | 0.04–1.0 | 5 |
| **Growth factor** | **Level in milk ($\mu$g/L)** | **Level in colostrum ($\mu$g/L)** |
| IGF-1[a] | <10 | 50–2000 |
| IGF-2 | <10 | 200–600 |

[a] IGF: Insulin-like growth factor.
Source: data fro Marnila and Korhonen (2002) and Pakkanen and Aalto (1997).

unequivocally determined. Curiously, despite tests on a large range of milks for varying purposes, bovine milk deficient in $\beta$-lactoglobulin has never been reported in the literature. This may imply an essential role in the milk of at least some species.

*$\alpha$-Lactalbumin*

$\alpha$-Lactalbumin is the second most abundant whey protein, and has a role in the synthesis of lactose. The protein is the source of several bioactive peptides (see later), but also has a potential bioactive function in its own right. HAMLET (human $\alpha$-lactalbumin made lethal to tumour cells) is a complex of partially refolded human $\alpha$-lactalbumin and oleic acid that kills tumour cells by an apoptosis-like mechanism (Svensson *et al.*, 2003). HAMLET is the result of a partial denaturation and re-folding of the protein in the presence of the fatty acid. Similarly BAMLET, the bovine $\alpha$-lactalbumin equivalent, shows the same activity (Svensson *et al.*, 2003). How this may develop in terms of functional foods or milks is unclear.

A further feature of $\alpha$-lactalbumin is its relatively high content of tryptophan: $\alpha$-lactalbumin contains four tryptophan residues, 5.2% by weight – a much higher level than is found in other milk proteins or most other food proteins. Tryptophan is a precursor of serotonin, and uptake of tryptophan by the brain for serotonin synthesis is considered to be important for sleep. To be effective, tryptophan must cross the blood–brain barrier. The transport system that takes tryptophan across this barrier is specific only for large neutral amino acids, so the ratio of tryptophan to total large neutral amino acids (Trp:LNAA) will determine its effectiveness. Clinical trials have shown that an evening meal enriched in $\alpha$-lactalbumin led to a significant increase in plasma Trp:LNAA, and improved morning alertness, most likely from improved sleep (Markus *et al.*, 2005).

Because the presence of $\alpha$-lactalbumin is so tightly coupled with lactose production, and consequently milk volume, it is unclear how an enriched milk might be usefully produced, although selection for the B variant of $\beta$-

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

lactoglobulin, which is expressed at a lower level than the A variant (Lum *et al.*, 1997), will increase the proportion of $\alpha$-lactalbumin in the whey protein fraction.

*Bioactive peptides*

Enzyme-mediated breakdown of milk proteins is known to give a wide range of bioactive milk peptides (see Korhonen and Pihlanto, 2006, for a recent review). Many of these peptides can be expected to be naturally formed in the gut during the digestive process, or formed during fermentative processes used in the dairy industry (such as the manufacture of yoghurt or cheese). Some of the main naturally-produced bioactive peptides are outlined in Table 3.2. The main known functionalities are:

- Effects on digestion (for example, the caseinophosphopeptides enhance calcium uptake)
- Potential effects on the nervous system (the casomorphins are possible receptor agonists; the casoxins antagonists) – although whether or how these peptides might have an effect beyond the gut is unclear
- Potential effects on the cardiovascular system (ACE inhibitors – inhibitors of angiotensin-converting enzyme – have antihypertensive activity, but again it is unclear how these might exert an effect beyond the gut)

**Table 3.2** Bioactive peptides from milk

| Origin of peptide | Biological effect | $IC_{50}$ ($\mu$M)[a] | Comment |
|---|---|---|---|
| *Casein-derived* | | | |
| $\alpha$-S1-Casein 23–34 | ACE inhibitor | 77 | Casokinin |
| $\alpha$-S1-Casein 66–74 | Ca binding | | Caseinophosphopeptide |
| $\alpha$-S1-Casein 90–96 | Opioid agonist | 12 | Also 90–95; $\alpha$S1 exorphin |
| $\alpha$-S1-Casein 194–199 | ACE inhibitor | 16 | $\alpha$S1 Immunocasokinin |
| | Immunostimulatory | 162 | |
| $\beta$-Casein 1–25 | Ca binding | | Caseinophosphopeptide |
| | Immunostimulatory | | |
| $\beta$-Casein 60–66 | Opioid agonist | 14 | Also 60–64, 60–70; |
| | Immunostimulatory | Variable | $\beta$-casomorphin-7 (5, 11) |
| $\kappa$-Casein 25–34 | Opioid antagonist | 50 | Casoxin-C |
| $\kappa$-Casein 33–38 | Opioid antagonist | 250 | Casoxin-6 |
| *Whey protein-derived* | | | |
| $\alpha$-Lactalbumin 50–53 | Opioid agonist | 300 | $\alpha$-Lactorphin |
| | ACE inhibitor | 733 | |
| $\alpha$-Lactalbumin 104–108 | ACE inhibitor | 77 | Lactokinin |
| $\beta$-Lactoglobulin 102–105 | Opioid agonist | 160 | $\beta$-Lactorphin |
| | ACE inhibitor | 172 | |
| $\beta$-Lactoglobulin 142–148 | ACE inhibitor | 43 | Lactokinin |

[a] $IC_{50}$: Concentration for 50% binding or effect (stimulation or inhibition).
Source: data from Pihlanto-Leppala (2002).

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

- Enhancement of the immune system through specific immunostimulatory peptides, mostly interacting with the gut immune system.

All of the listed peptides are natural products of digestion, and arise from major milk proteins. It is, in principle, possible to enrich or deplete individual milk proteins. In practice, the only significant and constant change in relative protein expression is due to the $\beta$-lactoglobulin A/B polymorphism, in which the A variant is expressed at higher levels due to an upstream mutation in a regulatory region (Lum *et al.*, 1997).

### 3.1.2 Lipids

There has been less focus on the possible functional properties of milk lipids than for the proteins and peptides. The high levels of saturated fat in milkfat and butter are viewed by many as undesirable for heart health, although the evidence for this is far from clear (see, for example, Poppitt *et al.*, 2002; Siri-Tarino *et al.*, 2010). Clearly a milkfat with a higher degree of unsaturated fatty acids would be desirable from a health point of view, as well as making butter more spreadable, because triglycerides with unsaturated fatty acids have lower melting points.

While polyunsaturated fatty acids cannot generally be considered bioactive, there are two classes of polyunsaturated fatty acids that are the subject of considerable interest, with good evidence of bioactivity: conjugated linoleic acids and $\omega$-3 long-chain polyunsaturated fatty acids.

IP Address: 129.132.208.100

*Conjugated linoleic acids*

The conjugated linoleic acids (CLAs) have been investigated for many years, with a range of bioactive effects observed, particularly protection against various forms of cancer in animal models (Parodi, 1997). The form of CLA found in milkfat is almost totally the *cis*-9, *trans*-11 isomer (Parodi, 1977), which has been associated with protective effects against some cancers in animal models (Kelley *et al.*, 2007) although not others. The chemically manufactured form of CLA (for example, that sold as Tonalin$^{TM}$) is largely a mixture of the *cis*-9, *trans*-11 isomer and the *trans*-10, *cis*-12 isomer. The latter isomer is also associated with protection against a range of cancers, although not always the same forms of cancer. It has become clear that the different isomers act through different pathways (Kelley *et al.*, 2007).

*$\omega$-3 Long-chain polyunsaturated fats*

The long-chain $\omega$-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are finding favour as being beneficial for heart health and mental health (see, for example, Kris-Etherton and Yu, 1997; Ruxton *et al.,* 2004; Garg *et al.*, 2007). Levels of these in milkfat are naturally low, and enrichment is most likely to be by addition of fish oils directly to the milk, usually in a microencapsulated form to mask the fishy flavour (e.g. Singh *et al.*, 2005).

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

*Milk polar lipids*

Milk contains significant amounts of polar lipids, particularly phosphatidyl choline, phosphatidyl ethanolamine and sphingomyelin, as well as smaller amounts of phosphatidyl inositol and cerebrosides (Fong *et al.*, 2006). These occur largely in the milkfat globule membrane, and fractionate during processing (such as butter making) into buttermilk and other by-products. These lipids, or their digestive products, have had a range of bioactive properties attributed to them, including cancer inhibition, cholesterol lowering, bactericidal effects against gut pathogens, and beneficial effects on a range of neurological conditions (Dewettinck *et al.*, 2008). None of these components is unique to milk, but the availability of buttermilk and other fractions enriched in milk fat globule membrane as by-products of normal dairy processing suggests these may be a useful source of functional ingredients. It is questionable whether it would be worth altering milk production specifically to enrich any or all of these components; however, there may be useful seasonal variations that could be considered.

### 3.1.3 Oligosaccharides

The predominant natural carbohydrate in milk, lactose, is not noted for any particular bioactivity. In human milk, lactose-containing oligosaccharides are a major component, comprising 5–10 g/l (Kunz and Rudloff, 2002). They contain significant amounts of N-acetyl glucosamine, N-acetylneuraminic acid and fucose, monosaccharides not found in large quantities in bovine milk. These oligosaccharides are thought to be important as prebiotics, promoting the development of beneficial intestinal flora, such as bifidobacteria, and also preventing the adhesion and colonisation of some undesirable bacterial species.

Bovine milk contains only trace amounts of oligosaccharides, although levels are somewhat higher in colostrum. During processing with lactase enzymes, it can form a range of galactooligosaccharides (GOS). The potential benefits of GOS as prebiotics are still being explored, although it is likely that oligosaccharides from non-milk sources will be a more efficient and economical alternative.

IP Address: 129.132.208.100

### 3.1.4 Minerals

Milk contains significant amounts of some essential elements, which have been discussed in Chapter 1. Whilst essential elements are nutritionally important, whether they can be considered as functional components is in most cases questionable. Ways to increase biologically important minerals in milk are discussed by Knowles *et al.* (2006) and the reader is referred to that publication for more information.

Calcium is the most important nutritional mineral in milk. Calcium secretion in milk is generally very tightly controlled (of necessity, to protect the bones of the lactating animal), and attempts at enhancement of milk calcium have generally not been successful.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

A possible truly functional milk is milk enhanced with selenium. Selenium is unusual in having a specific codon in mRNA that specifies its insertion into proteins as selenocysteine. Selenium is widely recognised as having an important protective effect against some cancers (Rayman, 2005), and many populations, particularly in Europe, are selenium-deficient. Selenium-enriched milk is thus seen as a desirable way of adding selenium to the diet.

### 3.1.5 Other small molecules

In addition to the above, milk contains a range of small organic molecules including small amounts of hormones and vitamins, as well as the possibility of metabolites originating from forage. The most noticeable of the latter is $\beta$-carotene, responsible for the yellow colour of milkfat from pasture-fed cows. It has been proposed that $\beta$-carotene might have protective effects against cancers (particularly lung cancer) and heart disease; however, extensive trials have demonstrated little effect on either (Omenn *et al.*, 1996).

Of particular interest from a bioactive point of view is melatonin, a natural bioactive that promotes sleep. As people age, their natural production of melatonin decreases (Sack *et al.*, 1986) and supplementation can be useful in treating resulting sleep disorders.

## 3.2 Milks with manipulated functional properties: production and application

IP Address: 129.132.208.100

Milk is a rich source of beneficial bioactive components as outlined in the previous section. In this section, we describe how the composition of milk can be manipulated on farm and by processing to enhance supposedly beneficial components. This section deals with milks that are enhanced by on-farm and/or processing interventions, but does not cover simple addition of bioactives from non-milk sources, nor are components covered that cannot reasonably be produced in milk at biologically significant levels.

There are several ways milk can be managed on farm to change composition, the principal ones being (Boland, 2003):

- Specific breeding to select for particular protein variants or breed characteristics leading to enhanced expression of particular components (particularly important for proteins). This approach requires reliable and cost-effective means of testing of large numbers of animals to identify elite animals for breeding purposes. Such tests are well established for the main genetic variants of the major milk proteins.
- Selection of elite animals from within very large herds (thousands of animals) to form sub-herds with modified composition – again requiring cost-effective tests for animal selection.
- Feeding to change the composition of milk – particularly with respect to milkfat. There is a large literature on this approach (Boland, 2003; Chilliard

© Woodhead Publishing Limited, 2010

Downloaded From Vet-eBooks.com

*et al.*, 2000), and manipulation of milkfat properties by this means is relatively straightforward.
- Selection of milk from particular parts of the breeding cycle (e.g. colostrum), season of the year or time of day (such as night milk for melatonin). This is based on well-understood cycles in milk production.

Most of these approaches are already in use for production of milk with enhanced properties for manufacturing (see, for example, Boland *et al.*, 2000). In some cases, specific interventions on farm can be used to produce special high-value milks, such as hyperimmune milks.

Processing can be used to change or enrich bioactive components. Enzymic processing can be used to develop bioactive peptides and milk-derived oligosaccharides, for example. Separation techniques are used to obtain enriched streams of desirable components such as lactoferrin and other bioactive proteins and peptides. A discussion of these is beyond the scope of this chapter.

### 3.2.1 Colostrum

Colostrum is the milk produced in the first few days after parturition. It differs considerably in composition from normal milk, notably in the presence of greatly elevated levels of immunoglobulins and of lactoferrin and a range of growth factors, principally insulin-like growth factors 1 and 2 (see Table 3.1).

Colostrum is obtained very simply by separately collecting the colostrum over the first 24 to 36 hours after parturition. Colostrum is not permitted to be mixed with normal milk in most jurisdictions, so if not collected for processing, it is still required to be collected separately and is usually fed to the newborn calves or other livestock.

IP Address: 129.132.208.100

Countries that have seasonally synchronised calving, such as New Zealand, are able to collect and process large volumes of colostrum during the spring calving season. It is also commercially viable to separately collect colostrum in large dairy farms of several thousand cattle, such as are found in the southwest of the United States. On such farms, there is a large enough number of cows calving each day to make collection and processing of significant volumes of colostrum possible.

Colostrum products are sold as tablets containing dried colostrum, dried colostrum powders or colostrum-based drinks. Because of the high immunoglobulin content of colostrum, drying processes are specialised to avoid protein denaturation. This is usually achieved by specialised low-heat spray drying, or by freeze drying. Commercial colostrum for human consumption is largely targeted to athletes, and treatment of or protection from diarrhoea, and general passive immune protection for the gut. Clinical trials of commercial colostrum products have shown success in treating AIDS-related gastrointestinal diseases (Florén *et al.*, 2006) and in treating diarrhoea in children (Patel and Rana, 2006).

Colostrum has long been claimed to be beneficial for athletes, and has been credited for the success of the Finnish cross-country Olympic ski team. Trials have shown increased levels of insulin-like growth factor and immunoglobulins

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

in athletes consuming colostrum products, and that the growth factors were not absorbed bovine growth factors (Mero *et al.*, 1997, 2002). Athletes often have impaired immune function following high-intensity exercise, and low doses of bovine colostrum protein concentrate supplement were found to beneficially modulate immune parameters during normal training and after an acute period of intense exercise (Shing *et al.*, 2007).

Colostrum products are also sold for young animal consumption, to protect against scours (Villadsen, 2005).

### 3.2.2 Hyperimmune milk

Hyperimmune milk is produced by immunising cows against specific antigens, generally in such a way that one or more of the immunoglobulins is over-produced in milk (or colostrum). Early products, produced in the early 1990s, were made by immunising lactating cows using an injection of a slow release formulation of a range of heat-killed bacteria (Beck, 1982; Beck and Stolle, 1989). This process resulted in a range of immunoglobulins against enteric bacteria, which were claimed to have anti-inflammatory properties (Beck, 1982), anti-hypertensive properties (Beck and Stolle, 1989; Sharpe *et al.*, 1994), cholesterol-lowering properties (Sharpe *et al.*, 1994), and efficacy in treatment of gastrointestinal disorders (Beck and Kotler, 1998). Hyperimmune milk has been available as a consumer product in some jurisdictions since the early 1990s, manufactured in the United States by Stolle Milk Biologics and in New Zealand by the New Zealand Dairy Board and subsequently Fonterra.

IP Address: 129.132.208.100

The predominant immunoglobulin in milk is IgG; however, there are good reasons to produce IgA for effectiveness in humans. In 1998, MucoVax was founded in the Netherlands to produce a hyperimmune milk-derived whey protein concentrate that has antibodies that are predominantly IgA, against the toxins of *Clostridium difficile*, a significant gastrointestinal pathogen (Thörig and Wouters-Wesseling, 2005). Animal and preclinical trials of this product showed promising results (Van Dissel *et al.*, 2005); however, full clinical results have yet to be reported. MucoVax and Nestlé are reported to be collaborating to develop hyperimmune milk-derived products for infant nutrition products specifically designed to prevent or lessen the severity of upper respiratory tract infections in young children. Technology to produce hyperimmune milk with elevated IgA has been developed by AgResearch in New Zealand (Hodgkinson and Hodgkinson, 2003), but this process does not appear to have been commercialised to date. A milk containing antibodies to *Candida albicans* has recently been described by this group (Hodgkinson *et al.*, 2007).

Although claims have been made about effectiveness of hyperimmune milk for a variety of conditions, strong clinical data are lacking. The case for passive immunity for treatment of gastrointestinal disorders is credible on a purely logical base. It is significant that no hyperimmune milk products are generally available with approved health claims.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

### 3.2.3 A2 milk

The 'A2 hypothesis' holds that milk containing the A1 variant of $\beta$-casein may have harmful effects on some individuals. The hypothesis arose from a consideration of dietary patterns and their linkage to type 1 diabetes (Elliott *et al.*, 1999), in particular the observation that when developing cultures change to a western diet and consume high amounts of dairy products, diabetes increases. Conversely, the Maasai, who consume large amounts of dairy, do not have high levels of diabetes. It turns out that the Maasai cattle are of the *Bos indicus* species, which does not have the A1 variant of $\beta$-casein; however, that variant occurs with a high frequency in *Bos taurus* cattle, particularly in the Holstein breed. Further studies included ecological studies comparing rates of occurrence of type 1 diabetes with the consumption of milk, corrected for the calculated proportion of the A1 variant (Elliott *et al.*, 1999), coupled with studies using mouse (Nod 1) and rat (BB) animal models for diabetes (Beales *et al.*, 2002).

In another ecological study, McLachlan (2001) drew a correlation between the consumption of the A1 variant milk and ischaemic heart disease. I had the opportunity to examine the underlying data for McLachlan's work and question its validity. It is noted that this work was published in an unrefereed journal.

A possible mechanism for the action of A1 milk has been proposed, based on the formation of the $\beta$-casomorphin peptide through hydrolysis of $\beta$-casein A1 during digestion. The peptide is proposed to be released in the gut and, when absorbed, acts as an agonist for the $\mu$ receptors, affecting brain and immune functions (Meisel and Fitzgerald, 2000). The A2 variant has a histidyl to prolyl substitution in position 67, which means the peptide cannot be easily released from hydrolysis by gut proteases. Difficulties with this mechanism include the likelihood of further breakdown of the $\beta$-casomorphin in the gut (animal experiments have not been able to show the presence of significant levels of the peptide in the gut during milk digestion), and the relative impermeability of the gut wall to peptides greater than tripeptides – hence no clear mechanism of absorption.

IP Address: 129.132.208.100

An independent comprehensive review of the literature has concluded that 'there is no convincing or even probable evidence that the A1 $\beta$-casein of cow milk has any adverse effect in humans' (Truswell, 2005).

A2 milk is available in supermarkets in Australia and parts of New Zealand. The method of production is simple: it involves screening cows for the $\beta$-casein variant and selecting only the A2A2 phenotype for milk supply. DNA tests are available for most milk protein polymorphisms, which means that calves and bulls can be selected for replacement and breeding purposes, respectively.

It has been argued that, because the A1 gene could easily be bred out of the herd, it should be removed as a precautionary measure. Such a line of reasoning is fallacious because, although the evidence against A2 milk is questionable, it is equally possible that good reasons may be found in future to prefer A1 milk over A2. The fact that both variants exist widely in the cow population suggests there is no deleterious effect of A1 milk on calves – if there were, evolution would have bred it out long before domestication.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

Supporters of the A2 hypothesis have also argued that the cost of changing to an all-A2 supply is minimal. This argument is naive. The operational cost of changing the milk supply over a period of time is relatively small, simply involving testing of animals and ongoing selection for only the A2A2 genotype. This, however, ignores the relatively enormous cost of the restriction on the gene pool. Modern breeding methods have been increasing the efficiency of milk production by the cow at a consistent rate of about 3% year on year for the last half-century and more. This is through selection of elite sires for breeding and intensive use of semen from those sires over large numbers of breeding cows. Most of these elite sires will not be homozygous for the A2 allele for $\beta$-casein, and restriction of breeding to the relatively small number of sires that are will set breeding and genetic gain back many years, with costs globally in the hundreds of millions of dollars a year. That cost might be justified if the case for A2 milk were proven, but on the basis of present evidence it is not.

The A2 hypothesis remains under review: a government-commissioned study in New Zealand has determined that the A2 hypothesis is not proven, and that more work needs to be done (Swinburne, 2004). In many other countries, the hypothesis is either ignored or not taken seriously.

### 3.2.4 Milk protein hydrolysates

Milk protein hydrolysates and whey protein hydrolysates are typically made by enzyme-catalysed hydrolysis of the relevant protein (usually pre-purified by membrane processes and/or ion exchange), followed by drying to a powder form.

Milk protein hydrolysates are produced both for nutritional purposes and for physical functionality. The primary use of nutritional milk protein (and more particularly whey protein) hydrolysates is in infant formula, where hydrolysis is a means to remove intact protein molecules, particularly $\beta$-lactoglobulin, that may cause an allergic reaction, while maintaining the nutritional value of milk. A further use of hydrolysed whey proteins is in sports nutrition, where whey proteins are prized because of their relatively high level of branched-chain amino acids. These amino acids are preferentially metabolised in muscle tissue, and are thus believed to provide more rapid recovery of muscle post exercise, and may have a role in signalling, leading to enhanced protein synthesis in muscle after exercise (Blomstrand *et al.*, 2006). Hydrolysed whey proteins are thought to be more rapidly digested than intact proteins, thus providing more rapid access to nutrition for the muscle.

### 3.2.5 Milk with modified fat composition

*Unsaturated fat*

In recent years considerable effort has gone into modification of cow feed to produce milk lipids with an increased degree of unsaturation. Saturation of mono- and polyunsaturated fatty acids occurs in the rumen, largely as a

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

detoxification mechanism for rumen bacteria, thus means to protect lipids during passage through the rumen have been explored. These range from the simple physical protection afforded by seed coats, such as that of canola or rapeseed, through to chemical modification by aldehydes, as seen in the Rumentek™ process (Scott *et al.*, 2001). The recent commercial release of an unsaturated fat-enhanced milk by Campina[1] in Europe attests to the efficacy and commercial viability of modification of milkfat by feeding.

Feeding of milkfat from cows fed modified, rumen-protected diets has been shown in clinical trials to have beneficial effects on serum cholesterol, particularly in lowering LDL-cholesterol while leaving HDL-cholesterol relatively unchanged (Noakes *et al.*, 1996; Poppitt *et al.*, 2002).

A DHA-enriched milk is sold in Canada under the name of 'Dairy Oh!™'. This is produced by dietary supplementation with fish meal (Wright *et al.*, 2003). The milk, introduced to the market in 2004, is claimed to contain 20 mg DHA per 250 ml serving for the homogenised milk and 10 mg DHA per 250 ml serving for the 1% fat and chocolate flavoured milks.

*Conjugated linoleic acid*

Dairy CLA is produced in the mammary gland by the desaturation of *trans*-vaccenic acid by the $\delta$-9 desaturase enzyme. There is also a microbial pathway that can occur in the rumen, but it is thought that the mammary pathway predominates. Levels of CLA in milk and dairy products are affected greatly by diet (Khanal and Olson, 2004; Dhiman *et al.*, 2005). Milkfat from pasture-fed cows typically contains around 1% CLA, compared with less than 0.5% in milk from grain-fed cows (Boland, 2003, and references therein). Levels are also influenced by breed, farm practice and individual animal genetics. A herd-to-herd variation of 0.8% to 2.2% CLA in milkfat from pasture-fed herds collected on the same day was reported in one study (MacGibbon *et al.*, 2001). These differences may relate to differences in levels of the desaturase, although how this occurs is far from clear (Moioli *et al.*, 2007).

Clinical and other trials have supported a range of health benefits from consumption of dairy CLA:

- The mechanisms by which CLA inhibits cancers are beginning to be understood, with the demonstration of inhibition of an oestrogen signalling pathway related to breast cancer (Tanmahasamut *et al.*, 2004).
- Clinical trials on blood lipids indicated a significant reduction in very low density lipoprotein triacylglycerols (VLDL-TAG), which would correspond to a 12.5% reduction in risk for coronary artery disease (Noone *et al.*, 2002).
- Recent animal studies have indicated an anti-inflammatory role for conditions such as asthma (Kanwar *et al.*, 2008).

1. See http://www.frieslandcampina.com/english/innovation/innovations/drinks-and-deserts/campina-milk-unsaturated-fatty-acids-outdoor-grazing-selected-farmers.aspx.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

Milk and dairy products specifically from pasture-fed cows, claimed to be high in CLA as a result, are marketed by a number of dairies in the United States, for example cheese sold under the 'Minnesota Farmstead' label, and 'Organic Valley Pasture Butter'. Outside the United States, public awareness of CLA is low and demand correspondingly weak; however, given that animal fats are the only significant natural source of these fatty acids in the diet, interest can be expected to increase as results of trials become clearer.

### 3.2.6 Selenium-enriched milk

Milk is relatively easily enriched in selenium by feeding cows a selenium supplement (Knowles *et al.*, 1999). These authors were able to increase milk selenium up to seven-fold, and observed that feeding of selenised yeast (an organic form of selenium) was more effective than feeding selenium salts. In New Zealand, AgResearch has recently announced two new technologies to simplify selenium enrichment of milk. One new technology is an injectable supplement for cows and sheep. The other is the Senrich$^{TM}$ bolus – a small capsule that is swallowed by a cow or sheep and slowly dissolves in the rumen over 8–16 weeks (AgResearch, 2007). Selenium-enriched milk protein is sold commercially in Australia by Tatura.

Animal trials comparing dairy-selenium with yeast-selenium in a mouse model for colorectal cancer have shown that dairy-selenium at 1 ppm significantly suppressed aberrant crypt foci and cancers, whereas yeast-selenium at an equivalent selenium intake had no effect. Dairy-selenium significantly reduced cell proliferation and frequency of *K-ras* mutations in aberrant crypt foci relative to an equivalent dose of selenium from yeast. Selenium-enriched milk protein isolate was thus seen as superior to selenised-yeast in terms of its bioavailability and capacity to suppress oncogenesis (Hu *et al.*, 2008).

Clinical trials with ileostomates have shown that selenium from enriched milk is efficiently absorbed by the body (Chen *et al.*, 2004); however, recent detailed clinical trials showed that thrombocyte glutathione peroxidase was specifically increased by short-term selenate supplementation, but not by short-term supplementation with organic selenium, including selenium-enriched milk. Short-term selenium supplementation did not appear to affect blood lipid markers or expression and activity of selected enzymes and a transcription factor involved in glutathione-mediated detoxification and anti-oxidation processes (Ravn-Haren *et al.*, 2008).

### 3.2.7 Milk with high melatonin levels

Melatonin is known to occur at low levels in milk that vary diurnally (Berthelot *et al.*, 1990) and it is possible to collect milk that has enriched levels of melatonin by separate collection of night milk. Animal studies have shown that milk melatonin concentration reflects serum melatonin concentration, with a short lag phase in any change (Eriksson *et al.*, 1998). Two methods for

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

production of melatonin-enriched milk are mentioned in the patent literature: the primary, and expected, method involves management of the exposure of cows to light (managing photoperiod and/or wavelength) and milking at the end of the dark period (Valtonen *et al.*, 2000; Gnann, 2007). Another approach attempts to capture cow variability and uses in-line monitoring of a marker for melatonin to selectively collect milk from cows producing high levels (Shiro and Fumiya, 2008). Experiments with magnetic and electric fields showed effects on melatonin levels in cow serum during the day photoperiod, but no effects on night-time levels (Rodriguez *et al.*, 2004), and effects on milk might be expected to be similar.

A clinical trial feeding 'night-time milk' to elderly subjects in care showed no significant differences in sleep patterns, but did observe a pattern of significantly increased activity in morning and evening (Valtonen *et al.*, 2005). Whether the observed effect is meaningful for the wider population is open to question, but the possibility of a sleep-promoting, melatonin-rich milk remains an interesting prospect.

Melatonin-enriched 'night-time milk' was first marketed by Ingman Dairy in Finland in 1999. The first melatonin-enriched milk in the UK was Slumber Bedtime milk, produced by Red Kite. 'Night-time milk' by St Helen Farms, claimed to contain high levels of melatonin, was launched in the UK in 2004 and was still available in 2006 in major UK supermarket chains; however, the website used to advertise this milk, www.night-time-milk.com, appears to be occupied by a squatter, suggesting the milk may no longer be commercially available. Other sleep-enhancing milks have been released in Ireland (Lullaby milk) and more recently in the United States (Dreamerz milk). It is possible that the latter milk may contain synthetic melatonin, as it is not a controlled substance in the United States and is relatively inexpensive. Night-time milks have also been reported from Latvia and Japan. A melatonin-enriched milk produced in Switzerland, 'Nachtmilch', was banned by regulators, who considered sleeplessness to be an illness, thus the milk was regarded as a medicine.

IP Address: 129.132.208.100

## 3.3 Conclusions and future trends

Milk contains a range of bioactive components with potentially beneficial effects. With continuing elucidation of these beneficial effects and their mechanisms, demand will increase and, eventually, label claims are a likelihood. There is increasing consumer awareness of the relationship between nutrition and health, with functional foods increasingly available as mainstream food lines in supermarkets. With the emergence of nutrigenomics, and awareness of personal nutritional needs, there will be increasing demand for functional milks and their products.

Development of new, more sophisticated processing, including processes that are less likely to denature proteins, and processes to recover enriched streams of specific bioactives, will lead to wider availability of milk-derived functional

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

foods and ingredients. These factors will require more attention to management of milk production and specialised milks with altered composition will become more common. New business models involving novel payment systems with routine testing and payment for bioactive components will need to be developed, as well as cost-effective and efficient methods of testing animals to enable selection of elite producers for production and for breeding (Boland *et al.*, 2000).

The Campina milk with increased polyunsaturated fat is an early example of consumer milks with modified composition becoming mainstream. Companies with a vertically integrated approach to dairy production and manufacturing, such as New Zealand's Synlait (www.synlait.co.nz), are showing leadership in development of new milks, and may point the way of the future.

## 3.4 Sources of further information and advice

All of the references below are useful.

- For general purposes, the *Encyclopaedia of Dairy Sciences* edited by H. Roginski, J.W. Fuquay and P.F. Fox (Academic Press, 2003) is particularly recommended.
- The special issue of the *International Dairy Journal* (vol. 16, no. 11) on technological and health aspects of bioactive components of milk has a series of useful papers.
- For general reading on milk proteins, volume 1 of the excellent series edited by Pat Fox: *Advanced Dairy Chemistry*, from Elsevier Applied Science (2003), is recommended.

IP Address: 129.132.208.100

## 3.5 Acknowledgements

I would like to thank Jeremy Hill and Alastair MacGibbon of Fonterra, Eric Kolver formerly of Synlait, and Paul Moughan and Harjinder Singh of the Riddet Institute for providing valuable criticism.

## 3.6 References

AGRESEARCH (2007) Selenium-enriched milk straight from the cow. *InTouch*, Issue 34, p. 1. AgResearch, Hamilton, New Zealand (available at http://www.agresearch.co.nz/publications/intouch/AgResearch_News_Dec2007.pdf).

BEALES, P.E., ELLIOTT, R.B., FLOHÉ, S., HILL, J.P., KOLB, H., POZZILLI, P., WANG, G.S., WASMUTH, H. and SCOTT, F.W. (2002) A multi-centre, blinded international trial of the effect of A1 and A2 $\beta$-casein variants on diabetes incidence in two rodent models of spontaneous Type I diabetes. *Diabetologia* **45**:1240–1246.

BECK, L.R. (1982) Method of obtaining an anti-inflammatory bovine milk. Patent EP0064103.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

BECK, L.R. and KOTLER, D.P. (1998) Use of hyperimmune milk product to treat gastrointestinal disorders. Canadian Patent 1339933.

BECK, L.R. and STOLLE, R.J. (1989) Antihypertensive hyperimmune milk, production, composition, and use. US Patent 4879110.

BERTHELOT, X., LAURENTIE, M., RAVAULT, J.P., FERNEY, J. and TOUTAIN, P.L. (1990) Circadian profile and production rate of melatonin in the cow. *Domest. Anim. Endocrinol*. **7**: 315–322.

BLOMSTRAND, E., ELIASSON, J., KARLSSON, H.K.R. and KOHNKE, R. (2006) Branched-chain amino acids activate key enzymes in protein synthesis after physical exercise. *J. Nutr*. **136**: 269S–273S.

BOLAND M. J. (2003) Influences on raw milk quality, in *Dairy Processing: Maximising Quality* (ed. G. Smit), Woodhead Publishing, Cambridge, pp. 42–67.

BOLAND, M.J., HILL, J.P. and O'CONNOR, P.J. (2000) Changing the milk supply to increase cheese yield: the Kaikoura experience, in *British Society of Animal Science Occasional Publication No. 25* (ed. R.E. Agnew, K.W. Agnew and A.M. Fearon), British Society of Animal Science, Edinburgh, pp. 305–316.

BOOTS J.-W. and FLORIS, R. (2006) Lactoperoxidase: from catalytic mechanism to practical applications. *Int. Dairy J.* **16**: 1272–1276.

CHEN, J., LINDMARK-MANSSON, H., DREVELIUS, M., TIDEHAG, P., HALLMANS, G., HERTERVIG, E., NILSSON, A. and AKESSON, B. (2004) Bioavailability of selenium from bovine milk as assessed in subjects with ileostomy. *Eur. J. Clin. Nutr.* **58**: 350–355.

CHILLIARD, Y., FERLAY, A., MANSBRIDGE, R.M. and DOREAU, M. (2000) Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated *trans* and conjugated fatty acids. *Ann. Zootech*. **49**: 181–205.

DEWETTINCK, K., ROMBAUT, R., THIENPONT, N., LE, T.T., MESSENS, K. and VAN CAMP, J. (2008) Nutritional and technological aspects of milk fat globule membrane material. *Int. Dairy J.* **18:** 436–457.

DHIMAN, T.R., NAM, S-H. and URE, A.L. (2005) Factors affecting conjugated linoleic acid content in milk and meat. *Crit. Rev. Food Sci. Nutr*. **45**: 463–482.

DIPLOCK, A.T., AGGETT, P.J., ASHWELL, M., BORNET, F., FERN, E.B. and ROBERFROID, M.B. (1999) Scientific concepts for functional foods in Europe. Consensus document. *Br. J. Nutr.* **81**: S1–S27.

ELLIOTT, R.B., HARRIS, D.P., HILL, J.P., BIBBY, N.J. and WASMUTH, H.E. (1999) Type I (insulin-dependent) diabetes mellitus and cow milk: casein variant consumption. *Diabetologia* **42**: 292–296.

ERIKSSON, L., VALTONEN, M., LAITINEN, J.T., PAKKANEN, M. and KAIKKONEN, M. (1998) Diurnal rhythm of melatonin in bovine milk: pharmacokinetics of exogenous melatonin in lactating cows and goats. *Acta Vet. Scand.* **39**: 301–310.

FLORÉN, C., CHINENYE, S., ELFSTRAND, L., HAGMAN, C. and IHSE, I. (2006) ColoPlus, a new product based on bovine colostrum, alleviates HIV-associated diarrhoea. *Scand. J. Gastroent.* **41**: 682–686.

FONG, B.Y., NORRIS, C.S. and MACGIBBON, A.K.H. (2006) Protein and lipid composition of bovine milk-fat globule membrane. *Int. Dairy J.* **17**: 275–288.

GARG, M.L., BLAKE, R.J., CLAYTON, E., MUNRO, I.A., MACDONALD, L., SINGH, H. and MOUGHAN, P.J. (2007) Consumption of an *n*-3 polyunsaturated fatty acid enriched dip modulates plasma lipid profile in subjects with diabetes type II. *Eur. J. Clinical Nutr.* **61:** 1312–1317.

GNANN, T. (2007) Method for the production of milk or milk products having a high melatonin content. Patent WO2007068361.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

HEINZMANN, A., BLATTMANN, S., SPUERGIN, P., FORSTER, J. and DEICHMANN, K.A. (1999) The recognition pattern of sequential B cell epitopes of beta-lactoglobulin does not vary with the clinical manifestations of cow's milk allergy. *Int. Arch. Allergy Immunol*. **120**: 280–286.

HODGKINSON, A.J. and HODGKINSON, S.C. (2003) Processes for production of immunoglobulin A in milk. US Patent 6616927.

HODGKINSON, A.J., CANNON, R.D., HOLMES, A.R., FISCHER, F.J. and WILLIX-PAYNE, D.J. (2007) Production from dairy cows of semi-industrial quantities of milk-protein concentrate (MPC) containing efficacious anti-*Candida albicans* IgA antibodies. *J. Dairy Res.* **74**: 269–275.

HU, Y., MCINTOSH, G.H., LE LEU, R.K., WOODMAN, R. and YOUNG, G.P. (2008) Suppression of colorectal oncogenesis by selenium-enriched milk proteins: apoptosis and K-ras mutations. *Cancer Research* **68**: 4936–4944.

KANWAR, R.K., MACGIBBON, A.K., BLACK, P.N., KANWAR, J.R., ROWAN, A., VALE, M. and KRISSANSEN, G.W. (2008) Bovine milk fat enriched in conjugated linoleic and vaccenic acids attenuates allergic airway disease in mice. *Clinical and Experimental Allergy*, **38**: 208–218.

KELLEY, N.S., HUBBARD, N.E. and ERICKSON, K.L. (2007) Conjugated linoleic acid isomers and cancer. *J. Nutr.* **137**: 2599–2607.

KHANAL, R.C. and OLSON, K.C. (2004) Factors affecting conjugated linoleic acid (CLA) content in milk, meat, and egg: a review. *Pakistan J. Nutr*. **3**: 82–98.

KNOWLES, S.O., GRACE, N.D., WURMS, K. and LEE, J. (1999) Significance of amount and form of dietary selenium on blood, milk, and casein selenium concentrations in grazing cows. *J. Dairy Sci*. **82**: 429–437.

KNOWLES, S.O., GRACE, N.D., KNIGHT, T.W., MCNABB, W.C. and LEE, J. (2006) Reasons and means for manipulating the micronutrient composition of milk from grazing dairy cattle. *Anim. Feed Sci. Technol.* **131**: 154–167.

KORHONEN, H. and PIHLANTO, A. (2006) Bioactive peptides: production and functionality. *Int. Dairy J*. **16**: 945–960.

KRIS-ETHERTON, P.K. and YU, S. (1997) Individual fatty acid effects on plasma lipids and lipoproteins: human studies. *Am. J. Clin. Nutr.* **65**: S1628–S1644.

KUNZ, C. and RUDLOFF, S. (2002) Health benefits of milk-derived carbohydrates. *Bull. Int. Dairy Fed*. **375**: 72–79.

LUM, L.S., DOVC, P. and MEDRANO, J.F. (1997) Polymorphisms of bovine $\beta$-lactoglobulin promoter and differences in the binding affinity of activator protein-2 transcription factor. *J. Dairy Sci.* **80**: 1389–1397.

MACGIBBON, A.K.H., VAN DER DOES, Y.E., FONG, B.F., ROBINSON, N.P. and THOMSON, N.A. (2001) Variations in the CLA content of New Zealand milkfat. *Aust. J. Dairy Technol.* **56**: 158.

MARKUS, C.R., JONKMAN, L.M., LAMMERS, J.H.C.M., DEUTZ, N.E.P., MESSER, M.H. and RIGTERING, N. (2005) Evening intake of $\alpha$-lactalbumin increases plasma tryptophan availability and improves morning alertness and brain measures of attention. *Am. J. Clin. Nutr.* **81**: 1026–1033.

MARNILA, P. and KORHONEN, H. (2002) Immunoglobulins, in *Encyclopaedia of Dairy Sciences* (ed. H. Roginski, W. Fuquay and P.F. Fox), Academic Press, London, pp. 1950–1956.

MCLACHLAN, C. (2001) $\beta$-Casein A1, ischaemic heart disease mortality, and other illnesses. *Medical Hypotheses* **56**: 262–272.

MEISEL, H. and FITZGERALD, R.J. (2000) Opioid peptides encrypted in intact milk protein sequences. *Brit. J. Nutr*. **84**: S27–S31.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

MERO, A., MIIKKULAINEN, H., RISKI, J., PAKKANEN, R., AALTO, J. and TAKALA, T. (1997) Effects of bovine colostrum on serum IGF-I, IgG, hormone, and saliva IgA during training. *J. Appl. Physiol.* **83**: 1144–1151.

MERO, A., KÄHKÖNEN, J., NYKÄNEN, T., PARVIAINEN, T., JOKINEN, I., TAKALA, T., NIKULA, T., RASI, S. and LEPPÄLUOTO, J. (2002) IGF-I, IgA, and IgG responses to bovine colostrum supplementation during training. *J. Appl. Physiol.* **93**: 732–739.

MOIOLI, B., CONTARINI, G., AVALLI, A., CATILLO, G., ORRÙ, L., DE MATTEIS, G., MASOERO, G. and NAPOLITANO, F. (2007) Effect of stearoyl-coenzyme A desaturase polymorphism on fatty acid composition of milk. *J. Dairy Sci*. **90**: 3553–3558.

NAOT, D., GREY, A., REID, I.R. and CORNISH, J. (2005) Lactoferrin – a novel bone growth factor. *Clin. Med. Res.* **3**: 93–101.

NOAKES, M., NESTEL, P.J. and CLIFTON, P.M. (1996) Modifying the fatty acid profile of dairy products through feedlot technology lowers plasma cholesterol of humans consuming the products. *Am. J. Clin. Nutr.* **63**: 42–46.

NOONE, E.J., ROCHE, H.M., NUGENT, A.P. and GIBNEY, M.J. (2002) The effect of dietary supplementation using isomeric blends of conjugated linoleic acid on lipid metabolism in healthy human subjects. *Brit. J. Nutr.* **88**: 243–251.

OMENN, G.S., GOODMAN, G.E., THORNQUIST, M.D., BALMES, J., CULLEN, M,R., GLASS, A., KEOGH, J.P., MEYSKENS, F.L., VALANIS, B., WILLIAMS, J.H., BARNHART, S. and HAMMAR, S. (1996) Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *New England J. Med.* **334**: 1150–1155.

ORSI, N. (2004) The antimicrobial activity of lactoferrin: current status and perspectives. *Biometals* **17**: 189–196.

PAKKANEN, R. and AALTO, J. (1997) Growth factors and antimicrobial factors of bovine colostrum. *Int. Dairy J*. **7**: 285–297.

PARODI, P. (1977) Conjugated octadecadienoic acids of milk fat. *J. Dairy Sci*. **60**: 1550–1553.

PARODI, P. (1997) Cows' milk fat components as potential anticarcinogenic agents. *J. Nutr*. **127**: 1055–1060.

PATEL, K. and RANA, R. (2006) Pedimune in recurrent respiratory infection and diarrhoea – The Indian experience – The PRIDE study. *Indian J. Pediatrics* **73**: 585–591.

PIHLANTO-LEPPALA, A. (2002) Bioactive peptides, in *Encyclopaedia of Dairy Sciences* (ed. H. Roginski, W. Fuquay and P.F. Fox), Academic Press, London, pp. 1961–1967.

POPPITT, S.D., KEOGH, G.F., MULVEY, T.B., MCARDLE, B.H., MACGIBBON, A.K.H. and COOPER, G.J.S. (2002) Lipid-lowering effects of a modified butter-fat: a controlled intervention trial in healthy men. *Eur. J. Clin. Nutr.* **56**: 64–71.

RAVN-HAREN, G., BÜGEL, S., KRATH, B.N., HOAC, T., STAGSTED, J., JØRGENSEN, K., BRESSON, J.R., LARSEN, E.H. and DRAGSTED, L.O. (2008) A short-term intervention trial with selenate, selenium-enriched yeast and selenium-enriched milk: effects on oxidative defence regulation. *Brit. J. Nutr.* **99**: 883–892.

RAYMAN, M.P. (2005) Selenium in cancer prevention: a review of the evidence and mechanism of action. *Proc. Nutr. Soc.* **64**: 527–542.

RODRIGUEZ, M., PETITCLERC, D., BURCHARD, J.F., NGUYEN, D.H. and BLOCK, E. (2004) Blood melatonin and prolactin concentrations in dairy cows exposed to 60 Hz electric and magnetic fields during 8 h photoperiods. *Bioelectromagnetics* **25**: 508–515.

RUXTON, C.H., REED, S.C., SIMPSON, M.J. and MILLINGTON, K.J. (2004) The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *J. Hum. Nutr. Diet* **17**: 449–459.

SACK, R.L., LEWY, A.J., ERB, D.L., VOLLMER, W.M. and SINGER, C.M. (1986) Human melatonin production decreases with age. *J. Pineal Res* **3**: 379–388.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

SCOTT, T.W., GULATI, S.K. and ASHES, J.R. (2001) Feed supplement. US Patent 6322827.

SHARPE, S.J., GAMBLE, G.D. and SHARPE, D.N. (1994) Cholesterol-lowering and blood pressure effects of immune milk. *Am. J. Clin. Nutr.* **59**: 929–934.

SHING, C.M., PEAKE, J., SUZUKI, K., OKUTSU, M., PEREIRA, R., STEVENSON, L., JENKINS, D.G. and COOMBES, J.S. (2007) Effects of bovine colostrum supplementation on immune variables in highly trained cyclists. *J. Appl. Physiol.* **102**: 1113–1122.

SHIRO, K. and FUMIYA, S. (2008) Milking system of livestock. Japan Patent JP2008022785.

SINGH, H., ZHU, X. and YE, M. (2005) Lipid encapsulation. Patents WO2006 115420, EP1876905.

SIRI-TARINO, P.W., SUN, Q., HU, F.B. and KRAUSS, R.M. (2010) Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. *Amer. J. Clin. Nutr.* Doi: 10.3945/ajcn.2009.27725.

SVENSSON, M., FAST, J., MASSBERG, A.-K., DURINGER, C., GUSTAFFSON, L., HALLGREN, O., BROOKS, C.L., BERLINER, L., LINSE, S. and SVANBORG, C. (2003) $\alpha$-Lactalbumin unfolding is not sufficient to cause apoptosis, but is required for the conversion to HAMLET (human $\alpha$-lactalbumin made lethal to tumor) cells. *Protein Science* **12**: 2794–2804.

SWINBURNE, B. (2004) Beta casein A1 and A2 in milk and human health: Report to New Zealand Food Safety Authority. http://www.nzfsa.govt.nz/policy-law/projects/a1-a2-milk/a1-a2-report.pdf.

TANMAHASAMUT, P., LIU, J., HENDRY, L.B. and SIDELL, N. (2004) Conjugated linoleic acid blocks estrogen signaling in human breast cancer cells. *J. Nutr.* **134**: 674–680.

THÖRIG, L. and WOUTERS-WESSELING, W. (2005) Bovine anti-CD whey protein concentrates (WPCs) derived from milk of immunised cows with specific biological activity as an ingredient for medical food. *Innovations in Food Technology* 27: 18–19.

TOUCH, V., HAYAKAWA, S., YAMADA, S. and KANEKO, S. (2004) Effects of a lactoperoxidase–thiocyanate–hydrogen peroxide system on *Salmonella enteritidis* in animal or vegetable foods. *Int. J. Food Microbiol*. **93**: 175–183.

TRUSWELL, A.S. (2005) The A2 milk case: a critical review. *Eur. J. Clin. Nutr*. **59**: 623–631.

VALTONEN, M., KANGAS, A.-P. and VOUTILAINEN, M. (2000) Method for producing melatonin rich milk. Patent WO/2001/001784.

VALTONEN, M., NISKANEN, L., KANGAS, A.-P. and KOSKINEN, T. (2005) Effect of melatonin-rich night-time milk on sleep and activity in elderly institutionalized subjects. *Nord. J. Psychiatry* **59**: 217–221.

VAN DISSEL, J.T., DE GROOT, N., HENSGENS, C.M.H., NUMAN, S., KUIPER, E.J., VELDKAMP, P. and VAN'T WOUT, J.J. (2005) Bovine antibody-enriched whey to aid in the prevention of a relapse of *Clostridium difficile*-associated diarrhoea: preclinical and preliminary clinical data. *J. Med. Microbiol.* **54**: 197–205.

VILLADSEN, J.K. (2005) Bovine colostrum – a lucrative solution in piglet rearing. *Feed Mix* **13**: 25–27

WAKABAYASHI, H., YAMAUCHI, K. and TAKASE, M. (2006) Lactoferrin research, technology and applications. *Int. Dairy J.* **16**: 1241–1251.

WRIGHT, T.C., HOLUB, B.J., HILL, A.R. and MCBRIDE, B.W. (2003) Effect of combinations of fish meal and feather meal on milk fatty acid content and nitrogen utilization in dairy cows. *J. Dairy Sci.* **86**: 861–869.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

# 4

# Understanding and preventing consumer milk microbial spoilage and chemical deterioration

**M. Heyndrickx, S. Marchand, V. De Jonghe, K. Smet, K. Coudijzer and J. De Block, Institute for Agricultural and Fisheries Research (ILVO), Belgium**

IP Address: 129.132.208.100

**Abstract:** This chapter gives a broad overview of the possible spoilage defects in consumer milk, being either of a microbiological nature (e.g. spore formers and *Pseudomonas* enzymes) or of a chemical nature (e.g. light-induced oxidation). As these defects can be quite specific for, e.g., pasteurised versus ultra high temperature (UHT) treated milk, a detailed description of the mechanisms of spoilage, the main factors (on farm or industry level) influencing this spoilage, and the methods (including emerging methods on farm or industry level) to prevent spoilage, are discussed for different types of consumer milk. Finally, some future trends and further reading advice are given.

**Key words:** microbiological and chemical spoilage of consumer milk, prevention of spoilage, spore formers, *Pseudomonas* spoilage enzymes, light-induced oxidation.

## 4.1 Introduction

The dairy sector is historically one of the first (if not the first) food sectors which introduced processing steps and chemical as well as microbiological criteria along the production chain to both safeguard and monitor the quality of the processed dairy product. This has been and still is being focused mainly on the elimination of pathogens through the pasteurisation process, combined with

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

cooling in the whole milk chain to prevent outgrowth of spoilage microbiota, or the application of ultra high temperature treatment (UHT) and industrial sterilisation to produce commercially sterile consumer milk, which can be stored at ambient temperature. Nevertheless, the economic impact of spoilage problems is becoming even more important due to the increasing international trade in food products and the high quality demands at the business-to-business as well as the consumer level. It is estimated for the whole food industry that economic losses due to food spoilage are enormous and that one-fourth of the world's food supply is lost through microbial spoilage only (in t'Veld, 1996). Food spoilage can be considered as any change (physical or insect damage, visible or invisible growth of microorganisms, changes in texture, development of off-flavours) which renders a product unacceptable for human consumption.

The most commonly used heat treatments nowadays to eliminate a lesser or greater part of the raw milk microbiota include high temperature/short time (HTST) pasteurisation, extended shelf-life (ESL) pasteurisation, ultra high temperature (UHT) treatment, and sterilisation. By definition, pasteurised milk has received a heat treatment sufficient to minimise possible health hazards arising from pathogenic microorganisms associated with the raw product as well as adverse chemical, physical and organoleptic changes (Lewis, 1994; Ryser, 2003). Now HTST pasteurisation, a mild and continuous heating process (73–76ºC for 15 s) (Lewis, 1994; Ryser, 2003), is the most commonly used form of pasteurisation. Since the endogenous milk enzyme, alkaline phosphatase (ALP), has comparable inactivation kinetics as those pathogens, ALP levels in pasteurised milk are used as the critical determinant of pasteurisation efficiency (Commission Regulation (EC), 2006).

IP Address: 129.132.208.100

Extended shelf-life (ESL) pasteurisation is mainly used in the USA and Canada when a particular shelf-life is required (Bylund, 1995). Traditional ESL technology in North America incorporates a high heat treatment of the product (125–138ºC for 2–4 s) and sometimes even a microfiltration step, which provides normal pasteurised product sensory characteristics, combined with ultraclean packaging, which includes a controlled filling environment and container sterilisation (Bylund, 1995). These practices allow an extended shelf-life for a further 30–40 days on top of the 2–16 days that is traditionally associated with HTST pasteurised products (Chapman *et al.*, 2009). Nevertheless, ESL products must still be kept in a well-refrigerated chain (<5ºC) during distribution and in retail stores, just like HTST pasteurised products, in order to be sold as a safe and good sensorial quality product for human consumption (Simon and Hansen, 2009).

In a major part of Europe (e.g. Belgium, France, Spain, Portugal), however, the centralisation of the dairy industry, increased competition among dairy companies and the inconveniences of maintaining an adequate cold chain have resulted in the development of UHT milk for ambient temperature distribution. UHT processing is a continuous process, which takes place in a closed system that prevents the product from being contaminated by airborne microorganisms. Heating at temperatures higher than 130ºC (usually 140–150ºC) for a holding period of a few seconds (usually 2–10 s) is applied, followed by aseptic

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

packaging to produce a 'commercially sterile' product with a 3–6 month shelf-life at ambient temperatures (Burton, 1988; Kosaric *et al.*, 1981; Mehta, 1980). To meet the legal requirements established by the European Union directive 1662/2006, the colony count at 30ºC of unopened packages after 15 days of incubation at 30ºC must be microbiologically stable (Commission Regulation (EC), 2006). The time–temperature combinations used in UHT systems are determined by the need to inactivate heat-resistant bacterial endospores, such as those of *Geobacillus stearothermophilus*, and the need to limit chemical changes (e.g. cooked flavour and loss of vitamins), which decrease the sensory and nutritional qualities of the product (Burton, 1988; Dogan *et al.*, 2009).

Sterilisation exposes milk to such a high temperature–time combination that effectively all microorganisms are killed. This is an advantage particularly in hot climates since sterilised milk can be stored, even at ambient temperatures, for long periods. Sterilisation at 115–120ºC for 15–40 minutes, after filling and sealing bottles, eliminates the need for aseptic handling of the product (Bylund, 1995). However, the disadvantage is that the milk has been exposed to high temperature for a long time and this, unfortunately, has deleterious effects in that the colour and taste of the milk and its vitamin content are all affected (Harding, 1995; Lewis, 1994).

## 4.2 Spoilage of pasteurised and extended shelf-life (ESL) milk

### 4.2.1 Microbiological spoilage

IP Address: 129.132.208.100

In the last decades, it has been especially the aerobic spore-forming bacteria belonging to the genus *Bacillus* and allied genera that have caused specific problems for the production of pasteurised fluid milk specifically. This is due to extended refrigerated storage of raw milk before processing on the farm and in the dairy, higher pasteurisation temperatures, reduction of post-pasteurisation contamination by principally Gram-negative organisms such as *Pseudomonas* spp., and prolonged shelf-life requirements of the consumer product (Meer *et al.*, 1991). A combination of specific attributes of the spores and of the resulting vegetative cells gives the aerobic spore former *Bacillus cereus* a huge advantage not only over non-spore formers but even over other spore formers, and explains why this organism is a specific threat for the dairy industry. Firstly, like all bacterial spores, *B. cereus* spores are resistant to heat and will thus survive food processing steps such as pasteurisation or thermisation, which eliminate or reduce vegetative microbial cells. de Vries (2006) reported decimal reduction times at a heating temperature of 95ºC ($D_{95}$-values) varying from around 5 to as high as 80 minutes for spores of naturally occurring *B. cereus* strains. As a result, the final product will be contaminated with spores with little or no competition from vegetative cells, which would otherwise outgrow *B. cereus*. Secondly, an important part of the *B. cereus* group strains are psychrotolerant or psychrotrophic (note that the latter term is linguistically not ideal because it means 'cold eating', but it is still used by the International Dairy Federation

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

(IDF) and will be used here also), i.e. growing at temperatures of $\leq$7ºC (Carlin *et al.*, 2006). Moreover, it is important to note that the majority of *B. cereus* strains are able to grow starting from 10ºC, which represents mild temperature abuse conditions (Guinebretière *et al.*, 2008). To indicate the importance of the psychrotrophic *B. cereus* group strains, a separate species, *Bacillus weihenstephanensis*, was proposed for them (Lechner *et al.*, 1998). It has been estimated that 25% of all shelf-life problems of pasteurised milk and cream in the USA are caused by psychrotrophic spore formers (Sorhaug and Stepaniak, 1997). The shelf-life of pasteurised milk can be determined as the average time in days for the psychrotrophs to reach $1 \times 10^7$ cfu/ml during storage at 6ºC.

The defects caused by psychrotrophic aerobic spore formers in pasteurised milk are quite similar to those of other spoilage bacteria, namely souring, off-flavours and/or structural defects caused by proteolytic, lipolytic and/or phospholipase enzymes (Meer *et al.*, 1991). The off-flavours can be bitter, putrid, unclean, stale, rancid, fruity, yeasty or sour (Washam *et al.*, 1977). Bitter flavour is caused by protease activity on the milk proteins, while rancid and fruity flavours are caused by lipases (see also Section 4.3.2). Some specific spore forming species have been associated in the past with specific flavour and other defects (reviewed by Meer *et al.*, 1991), but a correct species identification in these older reports is questionable because of the use of (what now appear as) inadequate biochemical identification techniques. Nevertheless, recent investigations confirmed *Paenibacillus polymyxa* as gas and acid producer from lactose in milk and thus potentially causing a sour, yeasty and gassy defect in milk (De Jonghe *et al.*, 2010). This organism has been found as the main Gram-positive spoilage organism (accounting for 38% of the milk spoilage) of Swedish and Norwegian pasteurised milk stored at 5 or 7ºC (Ternström *et al.*, 1993). The structural defects are sweet curdling and bitty cream, which are both caused mainly by psychrotrophic strains of the *B. cereus* group, although sweet curdling may also be caused by other aerobic spore formers amongst which is the *Bacillus subtilis* group. Bitty or broken cream is characterised by fat destabilisation. The *B. cereus* group (almost) exclusively produces lecithinase or phospholipase C, which hydrolyses lecithin, the phospholipid included in the membranes round the fat globules, into diglyceride and phosphorylcholine (see also Section 4.3.2). The membranes of the fat globules are split, resulting in an unstable fat emulsion often appearing in the form of flocks or lumps floating on the surface of the milk or cream, especially when added to a hot beverage. Further breakdown of the choline into trimethylamine will result in a fishy smell and taste. Sweet curdling of milk is caused by proteinases. The first sign is the appearance of small buttons or 'pellicles' on the bottom of the container, which may be unnoticed by the consumer. Upon further storage (especially at >7ºC), a curd formation may appear over the entire bottom surface of the container. This defect is rather common and affects more containers upon prolonged refrigerated storage. A higher pasteurisation temperature increases the germination rate of *B. cereus* and accelerates the formation of these two milk defects (Hanson *et al.*, 2005).

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

In German extended shelf-life (ESL) milk produced by high heat treatment (127ºC for 5 s) and not packaged in a sterile environment, spoilage microbiota were found to be dominated by Gram-positive recontaminants, most of which were non-spore formers that grow slowly at low temperatures (coryneforms, enterococci, micrococci, staphylococci) (Mayr *et al.*, 2004). The composition of this recontaminating microbiota and the shelf-life of the ESL milk was found to be most unpredictable. However, as there exist many types of ESL milk, these observations may not be generalised.

### 4.2.2 Chemical deterioration

*Light-induced deterioration*

The effect of light is recognised as one of the major causes of deterioration of milk quality. Off-flavours developing in milk exposed to light derive from oxidative processes, principally involving changes in proteins and lipids. Light-induced flavour has two distinct components. One is described as 'burnt feathers', 'activated flavour' or 'sunlight flavour', which develops rapidly (first 2–3 days of storage) and is due to protein degradation. Both methional and dimethyldisulfide have been assigned to this off-flavour (Skibsted, 2000). No evidence indicates that losses following light exposure occur in the essential amino acids supplied by milk protein, but light-induced protein degradation leading to these off-flavours is an important problem with respect to consumer acceptability (Dimick and Kilara, 1983). The other light-induced off-flavour is characterised as 'metallic' or 'cardboard-like', develops two or three days later and is due to the volatile carbonyl components typical of lipid oxidation. Lipid oxidation in milk leads to the formation of carbonyl compounds. Since many of these carbonyl compounds are organically detectable at ppb levels, only a small amount of lipid oxidation needs to occur before flavour problems arise. The oxidation of the lipids requires $O_2$ and a suitable oxidation catalyst. The most important catalysts of lipid oxidation in market milk in practice are light and Cu (Schröder, 1983).

IP Address: 129.132.208.100

*Copper-induced oxidation*

Copper content and ascorbic acid oxidation are key factors in the development of oxidised flavour. Copper ions act as a potent catalyst in free radical chain lipid oxidation, even if present in only minute concentrations. Milk with a high proportion of its native copper associated with the milk fat globular membrane (MFGM) can give rise almost immediately to oxidative flavour defects, even before storage. In fluid milk products, the substrate for oxidation is largely, if not entirely, phospholipids with the production of volatile carbonyl compounds (Jenq *et al.*, 1988). The realisation of the predominant role of $Cu^{2+}$ in the promotion of lipid oxidation in milk has resulted in the rigorous avoidance of $Cu^{2+}$-containing metals or materials in the dairy plant (Dunkley and Franke, 1967).

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

*Vitamin-linked deterioration*

Riboflavin (vitamin $B_2$) plays an important role in light-induced oxidation. Riboflavin is a natural constituent of milk and is not altered by pasteurisation. It is an important agent in the development of both the activated and oxidised off-flavours induced by light. The vitamin is very photosensitive and its light-induced degradation follows first-order kinetics and is temperature dependent (Allen and Parks, 1979; Sattar *et al.*, 1977). Riboflavin can generate singlet oxygen ($^1O_2$), which can indirectly react with polyunsaturated fatty acids to give hydroperoxides and thereby promote light-induced lipid oxidation. This singlet oxygen is also responsible for the oxidation of vitamin D (King and Min, 1998). Also the photolysis of ascorbic acid is related to the photodegradation of riboflavin since it acts as a photosensitiser for the destruction of ascorbic acid (Allen and Joseph, 1985).

Many studies have demonstrated the important role of $Cu^{2+}$ and $O_2$ concentration in ascorbic acid (vitamin C) oxidation, which has been linked with the development of oxidised flavours. Numerous studies on the possible antioxidant or pro-oxidant effects of ascorbic acid have not succeeded in defining the complex role of ascorbate in the oxidative stability of milk. Ascorbate does also protect riboflavin against degradation by activated oxygen species (Lee *et al.*, 1998).

Milk is an important source of vitamin A. Vitamin A or carotenoids as vitamin precursors are sensitive to light and may isomerise or photooxidise and are also affected by the presence of riboflavin (Jung *et al.*, 1998). Oxidation products like beta-ionone with very low odour thresholds may be formed. Carotene, the precursor of vitamin A, has also been linked with oxidative stability of milk. Carotene destruction requires $O_2$ and also involves the simultaneous oxidation of the fat in which the carotene is dispersed. Beta-carotene is a very effective quencher of $^1O_2$.

Tocopherol is also correlated with susceptibility to oxidative deterioration because of its ability to inhibit free radical chain initiation and propagation. Tocopherols are easily oxidised.

IP Address: 129.132.208.100

### 4.2.3 Factors influencing the risk of spoilage

In 56% of samples of Danish pasteurised milk cartons, *B. cereus* was found and both prevalence and counts per ml (after storage at 7ºC for 8 days) were significantly higher in the summer than in the winter (Larsen and Jorgensen, 1997). A similar seasonal effect on counts or prevalence of the *B. cereus* group in pasteurised milk was found in recent studies in Poland and China, respectively (Bartoszewiez *et al.*, 2008; Zhou *et al.*, 2008). During the last decades, several possible contamination routes for *B. cereus* in pasteurised milk have been described with either raw milk or post-pasteurisation contamination of the pasteurised milk being the principal source (Fig. 4.1). *B. cereus* levels in raw milk are usually very low (e.g. average of 1.2 log spores/litre, Vissers *et al.*, 2007a). Raw milk in the farm tank is contaminated with *B. cereus* spores via the

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

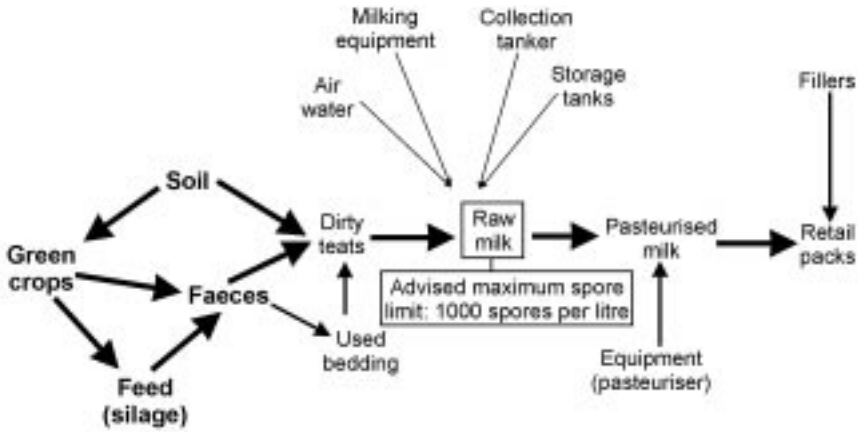



**Fig. 4.1** Sources of *Bacillus cereus* spores in raw and pasteurised milk. The relative importance of sources and contamination routes is indicated by the size of type and arrows.

exterior of the cow's teats and through improperly cleaned milking equipment. Contamination of the exterior of the teats occurs when teats are contaminated with dirt: during the grazing season of the cows, this dirt is mainly soil, while during the housing season of the cows, attached dirt is mainly faeces and bedding material. During milking, this dirt is rinsed off and spores present in the dirt can contaminate the raw milk. Soil is frequently highly contaminated with *B. cereus* (65–100%) and can contain up to $10^6$ *B. cereus* spores/g, but high variations (up to 3 log) can occur between sampling sites or times (Slaghuis *et al.*, 1997).

Highly variable levels of *B. cereus* spores (from below the detection limit up to $10^5$ cfu/g) can be present in faeces in different samples, but the average concentration seems to be around 2 log cfu/g (Wu *et al.*, 2007). *B. cereus* spores present in faeces probably originate from feed and (indirectly) from soil. By molecular typing, silage has been shown to be a significant source of contamination of raw milk with spores, including *B. cereus* spores, which may occur in levels up to $10^4$ cfu/g. From the moment the silage is used for feeding, the silage becomes exposed to air and aerobic deterioration takes place, which is initiated by acid-tolerant yeasts. During the later stages of aerobic spoilage of silage, growth of aerobic spore formers (including *B. cereus*) occurs, leading to elevated levels of spores (especially in the surface layers) compared to the initial levels on the green crops. Used bedding material may contain high levels of *B. cereus* spores, up to $10^6$ cfu/g, which indicates that there is active growth and sporulation, especially in the back of deep sawdust beds that come into contact with the udders (Magnusson *et al.*, 2007). From a survey on 24 farms in the Netherlands (none of them exceeding the maximum spore limit in the farm tank milk; see Section 4.2.4), it was found that transmission of *B. cereus* spores to farm tank milk occurred predominantly via faeces (and thus via mixed silage)

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

the whole year round (Vissers *et al.*, 2007a). Nevertheless, the authors pointed to the fact that under certain circumstances, especially wet conditions, transmission of substantial amounts of soil (>1 mg/litre milk) can occur, leading to spore levels in milk exceeding the maximum spore limit. Hence, for the dairy industry, control of the soil contamination pathway is most important. However, in some countries like China with an unbalanced distribution of raw milk resources, milk powder may be used to produce reconstituted milk in seasons when raw milk production is lowest and purchase prices are highest. As dried milk products contain fewer *B. cereus*, this may explain the lower prevalence of the *B. cereus* group in pasteurised milk in autumn in China (Zhou *et al.*, 2008).

The ability of *B. cereus* spores to adhere and act as an initiation stage for biofilm formation on a wide variety of materials commonly encountered in food processing plants is well known (Faille *et al.*, 2001; Peng *et al.*, 2001). The strong adhesion properties of spores of *B. cereus* have been attributed to the hydrophobic character of the exosporium (Faille *et al.*, 2001; Peng *et al.*, 2001), which varies from strain to strain (Tauveron *et al.*, 2006), and to the presence of appendages on the surface of the spores (Vanloosdrecht *et al.*, 1989). Thick biofilms of *B. cereus* developed on stainless steel coupons at the air–liquid interface, while biofilm formation was much lower in submerged systems (Wijman *et al.*, 2007). This suggests that *B. cereus* biofilms develop particularly in partly filled industrial storage and piping systems and that these biofilms act as a niche for formation of spores, which can be subsequently released by dispersal in the food production system. Spores embedded in biofilms are protected against disinfectants, such as chlorine, chlorine dioxide and a peroxyacetic acid-based sanitiser (Ryu and Beuchat, 2005). In some dairies, persistent silo tank contaminations, heat exchange equipment contamination or post-pasteurisation contamination are important sources of *B. cereus* (te Giffel *et al.*, 1996a, 1996b). For pasteurised and ESL milk, the filling machine has been shown to be the main source of recontamination with the filler nozzles, aerosols and the water at the bottom of the filling machine being of particular concern (Rysstad and Kolstad, 2006).

IP Address: 129.132.208.100

The photochemical reactions are influenced by many factors such as light source, wavelength, intensity, exposure time and temperature as well as the influence of the container material characteristics (Dimick, 1973). A detailed review on the influence of light transmittance of packaging materials on the shelf-life of milk and dairy products was given by Bosset *et al.* (1993).

### 4.2.4 Current methods to prevent spoilage

Refrigerated storage, enclosed pipeline milk systems, better sanitary design of equipment, better farm hygiene and more effective cleaning-in-place (CIP) systems have provided the opportunity for farms to produce raw milk with less microbial contamination (Barbano *et al.*, 2006). In the Netherlands, a maximum *B. cereus* spore limit in farm tank milk of 3 log spores/litre is necessary to

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

achieve a shelf-life of pasteurised milk of at least 7 days (Vissers *et al.*, 2007a). Using a predictive modelling approach, it was found that during the grazing period, a 99% reduction of the average concentration of *B. cereus* spores in raw milk could be achieved by minimisation of contamination of teats with soil and optimisation of teat cleaning efficiency at all farms in the Netherlands (Vissers *et al.*, 2007b). Another control option would be to house cows during summer on damp and rainy days when the concentration of *B. cereus* spores in soil is too high. During the housing period, care must be taken that the initial spore concentration in feeds is <3 log cfu/g and that the pH of the ration offered to the cows is below 5. To prevent outgrowth of spores in silage, special cultures of lactic acid bacteria or chemical additives can be applied to improve aerobic stability of the silage (Driehuis and Oude Elferink, 2000).

Pasteurised milk should preferably be stored during its whole shelf-life (including transport) at temperatures below 7ºC, because only a minority of the *B. cereus* strains grow at temperatures below 6ºC, while at 7ºC the generation time of psychrotrophic *B. cereus* in naturally contaminated pasteurised milk is in the range of 8 to >24 h (Larsen and Jorgensen, 1999). In the USA, pasteurised milk has a very long shelf-life of over 20 days because of a very good cold chain (Rysstad and Kolstad, 2006). A rule of thumb is that for every 2ºC increase of storage temperature, the shelf-life of pasteurised milk is reduced by 50%.

When these guidelines cannot be met and/or a longer refrigerated shelf-life is required than the traditional 7–14 days associated with regular HTST pasteurised milk, increasing pasteurisation regimes (e.g. ultra-pasteurisation) and/or a microfiltration step will be necessary to prevent product spoilage (Goff and Griffiths, 2006). Methods such as bactofugation and microfiltration can increase shelf-life of the milk in a good cold chain ($\leq$6ºC). The principle of microfiltration is to remove bacterial cells and spores from the milk mechanically using membrane processing with pore sizes bigger than in the cases of reverse osmosis and ultrafiltration (Maubois, 1997; Rysstad and Kolstad, 2006). Microfiltered milk is marketed in several countries as more 'pure' and 'natural' than standard heat-treated milk and has achieved a higher price as a branded product (Rysstad and Kolstad, 2006). In a suboptimal cold chain ($\geq$7ºC), on the other hand, a refrigerated shelf-life of 3–6 weeks can only be achieved by high-temperature or ultra-pasteurisation. This technology combines high-temperature heating with a short and controlled heating time, resulting in a good kill rate and acceptable to excellent sensory properties of the product (Rysstad and Kolstad, 2006).

To prevent spoilage it is important to reduce post-processing contamination (PPC) by adequate cleaning and disinfection of equipment and packaging materials and by the use of aseptic filling (Reij and Den Aantrekker, 2004; Rysstad and Kolstad, 2006). European legislation requires that handling, preparation, processing and packaging of food are done hygienically, with hygienic machinery in hygienic premises (Commission Directive, 1992). It is, however, left to the industry to decide how to comply with these requirements (Rysstad and Kolstad, 2006). In general the production of good quality foods is based on

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

the implementation of general preventive measures such as Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP). Significant specific hazards are addressed applying the Hazard Analysis Critical Control Point (HACCP) principles developed about 30 years ago (Reij and Den Aantrekker, 2004; Sandrou and Arvanitoyannis, 2000). Nevertheless, verification of the cleaning effect should be regarded as an essential part of cleaning operations (Reij and Den Aantrekker, 2004).

Adequate cleaning is of paramount importance before the use of sanitisers: besides the standard alkali/acid wash, also proteolytic enzyme-based cleaning systems may be used. Caustic (NaOH) chlorine washes can be very effective in removing well-established biofilms by breaking down the polysaccharide matrix, rather than inactivating the microorganisms (Brooks and Flint, 2008). After cleaning, sanitisers with sporicidal activity (i.e. killing spores) should be used. It is important to know that a number of bactericidal chemical compounds such as glutaraldehyde, formaldehyde, chlorine, iodine, acids and alkalis, hydrogen peroxide, peroxy acids, ethylene oxide, $\beta$-propionolactone and ozone are also sporicidal, but at much higher concentrations and longer contact times than for bactericidal action. Other compounds such as quaternary ammonia compounds, organic acids and alcohols are only sporistatic agents (i.e. preventing germination/outgrowth of spores). Also, it should be noted that commercial disinfectants, used under manufacturer-specified conditions, are less active on contaminated surfaces than is generally acknowledged (Sagripanti and Bonifacino, 1999) and that higher than recommended concentrations of sanitisers are usually required to effectively reduce attached bacterial populations on surfaces (Peta *et al.*, 2003). In aseptic filling machines for ESL milk, hydrogen peroxide is the predominant agent for sterilisation of packaging materials, usually at 35% concentration in combination with heat or at lower concentration in combination with UV-C light. For plastic bottles, peracetic acid in combination with hydrogen peroxide, followed by rinsing with sterile water, is used (Rysstad and Kolstad, 2006).

To prevent or reduce chemical spoilage it is recommended that the packaging material offer at least partial protection against light induced photo-oxidation. In this respect it is recommended that the maximum permissible light transmission of a packaging material should be 8% at 500 nm and 2% at 400 nm (Rysstad and Kolstad, 2006). Transparent packaging materials, like glass or plastic, offer only minimal protection against harmful light, whereas paperboard material gives very good light protection. The best protection is provided by ALU-foil paperboard that has a 0% transmission of light (Rysstad and Kolstad, 2006). It should be noted, however, that oxidative reactions (auto-oxidation) were reported to take place in milk packaged even in coated paperboard cartons, which were found to be more or less permeable to oxygen (Ryssstad *et al.*, 1998; Schröder *et al.*, 1985). It is proposed that multilayer (white pigment/black pigment/white pigment) pigmented and monolayer white pigmented HDPE 550–600 $\mu$m in thickness or pigmented PET bottles with a UV blocking agent may be used as possible alternatives to the coated paperboard carton for fresh

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

milk packaging (Moyssiadi *et al.*, 2004; Papachristou *et al.*, 2006a, 2006b; Zygoura *et al.*, 2004).

### 4.2.5 Emerging methods to prevent spoilage

The efficacy of CIP procedures on *B. cereus* spore viability was demonstrated to be greatly improved when a germinant mixture of L-alanine and inosine was used prior to the cleaning (Hornstra *et al.*, 2007). Dormant spores are 33–48% more adhesive than germinating spores (Anderson and Rönner, 1998), an observation that would also support the intentional germination of the spores prior to cleaning to increase the level of cleaning attained. There are several emerging systems to control biofilm formation (Brooks and Flint, 2008). Air-injected CIP systems, i.e. a turbulent two-phase flow system, are already used in milking systems to increase the cleaning efficiency. Stainless steel surfaces can be modified to reduce microbial colonisation.

Pressurised $CO_2$ as a potential non-thermal method for microbial reduction in raw foods and food products has gained interest, particularly within the past decade. It is illustrated that greater microbial lethality can be achieved in raw milk treated with supercritical phase $CO_2$ than with subcritical $CO_2$ and that the effects of supercritical $CO_2$ can be enhanced by increasing pressure and temperature (Werner and Hotchkiss, 2006). Supercritical $CO_2$ treatment using a flow-through high-pressure $CO_2$ processing unit can achieve bacterial reductions equal to or greater than those achieved by a range of possible pasteurisation treatments. Addition of $CO_2$ to raw bulk tank milk during cold storage prior to processing can significantly improve and extend the shelf-life of pasteurised milk. $CO_2$ addition could be a low-cost means to improve milk quality where low temperature refrigeration is inadequate. Additionally, it can enable longer-distance transport of fluid raw milk. Existing vacuum technology can be applied for $CO_2$ removal from raw milk immediately prior to pasteurisation. Although the process seems very promising for vegetative cells, spores are currently not affected by this treatment (Werner and Hotchkiss, 2006).

IP Address: 129.132.208.100

Ohmic heating or electroheating is a process in which an electrical current passes directly through pasteurised milk, resulting in the virtual destruction of all bacteria present by flash heating (Fillaudeau *et al.*, 2006; Sun *et al.*, 2008). Ohmic heating could be effectively used to pasteurise milk with no additional protein denaturation (Sun *et al.*, 2008). For commercial producers, ohmic heating also may present relatively few regulatory hurdles since this technique provides heat-sterilisation at temperatures identical to those of UHT processed milk (Dairy Management Inc., 2001). These alternative heat treatments (ohmic heating, radiofrequency and others) as well as non-thermal methods, such as high-pressure processing, electroporation or pulsed electric field treatment, have not been commercialised to a large extent, but for ESL milk one can expect that elements and/or combinations of such methods may lead in the future towards new, high quality milk products.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

## 4.3 Spoilage of ultra high temperature (UHT) and sterilised milk

### 4.3.1 Microbiological contamination and spoilage of UHT and sterilised milk

The (increasing) tolerance, adaptation or resistance of spores of specific spore forming species to conditions or treatments which were presumed to stop growth (low temperatures and low pH), or to inactivate all living material, such as UHT and commercial sterilisation is of more recent concern. In UHT-processed or sterilised milk, virtually all microorganisms including spores are killed. Both products are 'commercially sterile' (i.e. not more than one potential spoiled 1 litre container per 1000 and usually 1 per 10,000 or less) and have a long shelf-life (more than 6 months) without refrigeration. Spoilage by recontamination during filling infrequently occurs and is mostly caused by proteolytic activity of some *Bacillus* species. Massive contaminations of entire commercial lots of UHT and sterilised milk with a 'highly heat resistant spore former', termed HHRS or HRS, were first reported in Italy and Austria in 1985, and in 1990 also in Germany (Hammer *et al.*, 1995). Contrary to post heat-treatment contamination, this problem seemed to be caused by survival of the UHT process by the HRS spores, which could be isolated from a bypass directly after the heating section of an indirect UHT heating device, and occurred more frequently in indirect UHT than in direct UHT processing. The problem subsequently spread to other countries both within and outside Europe (Guillaume-Gentil *et al.*, 2002; Hammer *et al.*, 1995). After its peak in the mid-1990s, this problem reverted to a more sporadic or a tenacious contamination problem in some dairies. Affected milk products include whole, skimmed, evaporated or reconstituted UHT milk, UHT cream and chocolate milk in different kinds of containers, and also milk powders (Hammer *et al.*, 1995; Klijn *et al.*, 1997).

The HRS organism was taxonomically described as the new species *Bacillus sporothermodurans* based on isolates solely from UHT milk (Pettersson *et al.*, 1996). It appears as small, pinpoint colonies on plate count agar incubated at 30ºC, usually reaching a maximum of $10^5$ vegetative cells and $10^3$ spores per ml milk after an incubation according to the previous EC regulation (Commission Directive, 1992). This contamination level far exceeds the former explicit sterility criterion stipulated in the EU regulation. Recently, higher bacterial loads in 37% of Italian contaminated UHT milk samples exceeding $10^5$ cfu $ml^{-1}$ have been reported (Montanari *et al.*, 2004). The current EU hygiene regulation is less explicit on this point, stipulating that no viable microorganisms or spores may grow in the UHT-treated milk when kept at ambient temperature in aseptic closed containers and that this product must remain microbiologically stable after incubation for 15 days at 30ºC (or 7 days at 55ºC) in closed containers (Commission Regulation (EC), 2006). Real spoilage defects in consumer milk are not commonly noticed but may occur as a (slight) pink colour change, off-flavours and coagulation, especially in containers with a low oxygen barrier (e.g. plastic bottles). Despite its rather poor growth characteristics in milk, UHT milk

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

can be regarded as a new ecological niche for *B. sporothermodurans* because of the lack of competition from other organisms in this product.

### 4.3.2 Impact of endogenous and bacterial spoilage enzymes on quality

*Proteolytic spoilage enzymes*

Gelation

Although UHT milk is bacteriologically stable for months at ambient temperatures, its shelf-life is often compromised by age gelation. This phenomenon increases milk viscosity during storage and eventually results in a loss of fluidity with the formation of a gel (Datta and Deeth, 2001). The gel is a three-dimensional protein network formed by the whey proteins, particularly $\beta$-lactoglobulin, interacting with $\kappa$-casein of the casein micelle. Age gelation is initiated by proteolytic activity originating from either native milk protease (plasmin) or bacterial proteases that survive the UHT treatment.

Hydrolysis of milk caseins by proteases results in a destabilisation of the casein micelle (Fig. 4.2) (Bylund, 1995). After heat treatment, interactions between $\kappa$-casein and $\beta$-lactoglobulin occur, resulting in the release of the $\beta$-lactoglobulin-$\kappa$-casein complex ($\beta\kappa$-complex) from the micelle. The released complex subsequently aggregates and forms the typical network of crosslinked proteins, which causes the milk to gel (Datta and Deeth, 2001, 2003). Due to the greater heat intensity involved in the indirect heating method, the proteases in indirectly heat-treated UHT milk are inactivated to a greater extent (Corradini and Pecchini, 1981). Manji *et al.* (1986) observed no gelation up to 182 days at any temperature (4, 22–25, 37ºC) and less protein breakdown in indirectly heat-treated milk.

IP Address: 129.132.208.100

Proteolysis

Instability of casein micelles and the appearance of bitter off-flavours in UHT milk are caused mainly by residual or reactivated heat-stable proteolytic enzymes. Peptides and amino acids are known to cause a variety of taste sensations. Hydrophobicity has been found to be essential for bitter taste and casein, with relative high hydrophobicity known to yield a large amount of bitter peptides (Adler-Nissen, 1986; Fox, 1981). Proteases can be native, as plasmin, or produced by psychrotrophic bacteria during refrigerated storage prior to heat treatment.

The heat-resistant endogenous protease plasmin

Plasmin, the principal indigenous protease in milk, is the primary agent of proteolysis in good quality milk (Bastian and Brown, 1996; Grufferty and Fox, 1988; Kelly and McSweeney, 2003). Plasmin is the active constituent of a complex enzyme system and is closely associated with the casein micelles, as is its zymogen plasminogen (Visser, 1981). Plasmin activators (PAs) are serine proteases responsible for the conversion of plasminogen to plasmin (Fang and Sandholm, 1995; Lu and Nielsen, 1993). The activity of plasmin in milk is regulated by plasmin inhibitors (PIs) and by inhibitors of the plasminogen

© Woodhead Publishing Limited, 2010

Downloaded From Vet-e-Books.com

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

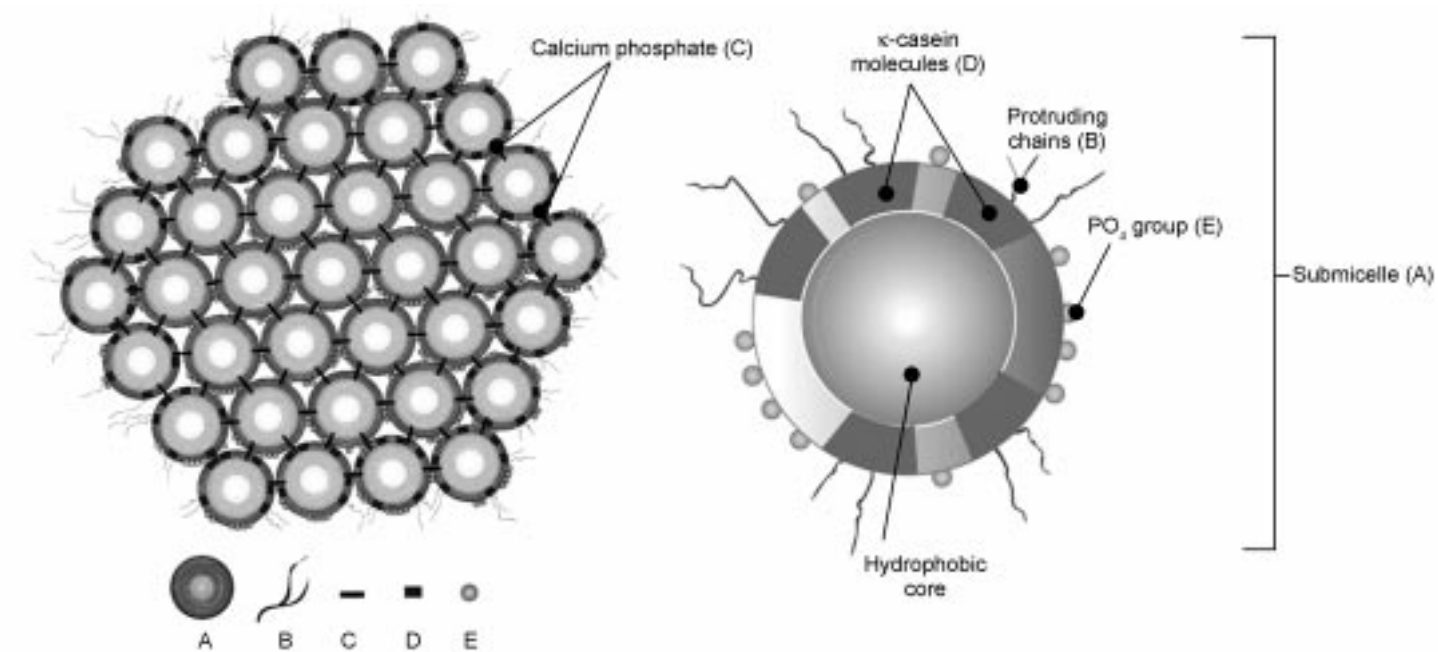



**Fig. 4.2** The casein micelle structure**.** A casein micelle consists of a complex of submicelles, in which $\alpha$-, $\beta$- and $\kappa$-casein are heterogeneously distributed. Calcium salts of $\alpha$-casein and $\beta$-casein are almost insoluble in water, while those of $\kappa$-casein are readily soluble. Due to the dominating localisation of $\kappa$-casein to the surface of the micelles, the solubility of calcium $\kappa$-caseinate prevails over the insolubility of the other two caseins in the micelles, and the whole micelle is soluble as a colloid. Adapted from *Dairy Processing Handbook* (Bylund, 1995).

Downloaded From Vet-eBooks.com

activators (PAIs), which are located in the serum phase of milk (Bastian and Brown, 1996; Grufferty and Fox, 1988). $\beta$-casein is the preferred substrate for plasmin and its hydrolysis results in the production of $\gamma$-caseins and proteose-peptones (Andrew and Alichanidis, 1983). $\alpha_{s2}$-casein is also readily hydrolysed by plasmin (Crudden *et al.*, 2005). However, plasmin does not hydrolyse $\kappa$-casein (Diaz *et al.*, 1996). Three members of the plasmin system (plasmin, plasminogen and PAs) have been reported to have very similar and relatively high heat-stabilities. This heat resistance may even result in a partial survival after UHT processing (Bastian and Brown, 1996; Fox and Kelly, 2006).

### Plasmin versus proteolytic enzymes of psychrotrophic bacteria

Proteolytic enzymes produced by psychrotrophic bacteria can spoil consumer UHT and sterilised milk and milk products because they retain their activity after even severe heat treatments, while the responsible producer organisms are completely inactivated. Enzymes of psychrotrophic bacteria, mainly pseudomonads, are typically produced in the late log or stationary growth phase (McKellar, 1989). The populations needed to cause detectable changes in UHT milk are usually in the range of $\geq 10^6$ cfu/ml raw milk (Law, 1979). Considerable spoilage enzyme synthesis occurs even at lower temperature, for example, production of extracellular protease by *Pseudomonas fluorescens* at 5ºC was 55% of that produced at 20ºC (McKellar, 1982). Furthermore, the enzymes remain active at temperatures well under their optimum temperature, for instance even at 2ºC for *P. fluorescens* (Braun *et al.*, 1999).

IP Address: 129.132.208.100

*Pseudomonas* proteases have many properties similar to the serralysin protease family of which the AprX protein of *P. aeruginosa* is the most extensively studied. The alkaline metalloprotease, which is encoded by the *aprX* gene, is believed to be responsible for the spoilage of milk. Although this protease gene is widespread over numerous *Pseudomonas* species (Chabeaud *et al.*, 2001; Chessa *et al.*, 2000; Duong *et al.*, 1992; Kawai *et al.*, 1999; Kumeta *et al.*, 1999; Liao and McCallus, 1998), the production process is still not completely understood and appears to be very complex. Quorum sensing (Juhas *et al.*, 2005; Liu *et al.*, 2007), temperature (Burger *et al.*, 2000; McKellar and Cholette, 1987; Nicodème *et al.*, 2005), iron content (McKellar, 1989; Woods *et al.*, 2001), aeration (Rowe and Gilmour, 1982) and phase variation (Chabeaud *et al.*, 2001; van den Broeck *et al.*, 2005) regulate and influence the production process at different levels.

*Pseudomonas fragi*, *P. lundensis* and *P. fluorescens* are most frequently involved in the spoilage of milk (Deeth *et al.*, 2002; Marchand *et al.*, 2009a, 2009b). Although the species *P. fluorescens* is very heterogeneous and exact identification remains controversial and difficult (Bossis *et al.*, 2000; Ercolini *et al.*, 2007; Marchand *et al.*, 2009a), most of the research on protease properties has focused on this species (Ching-hsing and McCallus, 1998; Kim *et al.*, 1997; Kumeta *et al.*, 1999). *P. fluorescens* produces only one protease, typically an alkaline zinc metalloprotease with a pH and temperature optimum of 6.5–8 and 37–45ºC, respectively (Fairbairn and Law, 1986; McKellar, 1989). In all

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com




**Fig. 4.3** Structure of the *aprX-lipA* operon in *P. fluorescens* B52. Adapted from McCarthy *et al.* (2004).

investigated proteases, activity decreased sharply at temperatures above the optimum, but all proteases for which data were available retained activity at 4ºC (McKellar, 1989). The alkaline metalloprotease gene (*aprX*) is usually located within operonic structures, which can contain genes encoding lipase (*lipA*), protease inhibitor (*inh*), a protease secretion apparatus (*aprDEF*) and auto-transporter proteins (*prtAB*) (McCarthy *et al.*, 2004; Woods *et al.*, 2001) (Fig. 4.3).

In contrast to plasmin, extracellular bacterial proteinases predominantly attack $\kappa$-casein with the formation of material similar to *para*-$\kappa$-casein (Datta and Deeth, 2001; Law, 1979), followed by extensive non-specific hydrolysis. $\beta$-Casein is also readily hydrolysed, while $\alpha_{s1}$-casein is slowly hydrolysed. In addition, the relative significance of proteolysis by milk plasmin and bacterial proteases in age gelation is somewhat blurred as there can be interaction between the two. Proteases produced by psychrotrophic bacteria may contribute to overall plasmin activity by acting as plasminogen activators to convert plasminogen into plasmin (Kohlmann *et al.*, 1991) and/or by disrupting the casein micelle, which causes release of plasmin into the milk serum, which in turn may enhance proteolysis and age gelation (Fajardo-Lira *et al.*, 2000).

*Lipolytic spoilage enzymes*

Psychrotrophic bacteria also produce lipolytic enzymes and represent different classes of enzymes, including esterases, true lipases and phospholipases. Lipases are enzymes that catalyse the hydrolysis of carboxyl ester bonds present in triglycerides (triacylglycerols), the major lipid component of milk. The products of this so-called 'lipolysis' are non-esterified free fatty acids (FFA), and partial glycerides (mono- and diglycerides) and in some cases even glycerol (Fig. 4.4). Lipases act at the lipid–water interface of emulsions of long-chain (>C10), insoluble triglycerides, while the related esterases act on esters of short chain fatty acids (<C10) and soluble esters, although lipases may also hydrolyse such substrates, as they display a much broader substrate range than the esterases (Jaeger *et al.*, 1994).

Esterases and lipases are serine hydrolases with a common structural motif at the active site (Gupta *et al.*, 2004), indicating a possible evolutionary link. Even though FFA produced due to lipolysis of milk fat are important for the development of cheese flavour (Hickey *et al.*, 2007), excessive lipolysis resulting from heat-resistant bacterial lipases can cause rancid off-flavours in consumer milk and cheeses with a long shelf-life. Several terms have been used to describe this flavour defect such as 'rancid', 'bitter', 'goaty', 'soapy', 'unclean' and 'butyric'. The flavour of whole milk with an elevated FFA level (>1.5 mmol/l) is unacceptable to most people (Deeth, 2006). The specific defect of rancidity occurs when the FFA involved is present in the acid form, resulting in a pH-

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

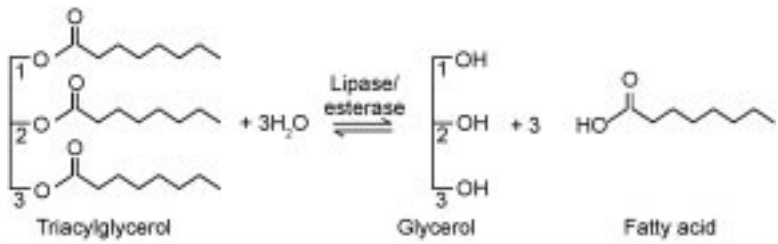



**Fig. 4.4** Enzymatic reaction of a lipase catalysing hydrolysis of a triacylglycerol substrate. The carbon atoms present in the glycerol core are annotated with numbers. The possibility of esterase or lipase activity depends on the length of the fatty acid chains in the triacylglycerol molecule.

dependent variability in intensity of the rancid off-flavour (IDF, 1991). However, the significance of the p$K_a$ values has relevance only to the extent of the solubility of the FFA in water that decreases very rapidly as the chain length increases, as shown in Table 4.1. Since homogenisation of the milk could help the fat and milk proteins to partially regain a protective interface (Mabbit, 1981), lipolytic spoilage of heat-treated milk is expected only in products which are stored for a rather long period of time, due to the action of trace amounts of bacterial lipolytic enzymes.

Some important lipase-producing bacterial genera in milk include *Bacillus*, *Pseudomonas* and *Burkholderia* (Gupta *et al.*, 2004). The largest family comprises the *Pseudomonas* true lipases, which are the most important lipases in spoilage of UHT products (Arpigny and Jaeger, 1999). Some *Pseudomonas* strains have been demonstrated to produce more than one lipase: the *lipB* gene encodes a secreted lipase that is solely responsible for the lipolytic phenotype of *P. fluorescens* strain C9, whereas *lipA* activity can only be detected intracellularly, showing an activity different from triglyceride hydrolysis (Woods *et al.*, 2001). Unlike the endogenous milk lipolytic enzyme (lipoprotein lipase or LPL), a pronounced specificity is rare among microbial lipases (Jensen, 1983),

IP Address: 129.132.208.100

**Table 4.1** Solubility of free fatty acids (FFA) in water at 20ºC (in grams of acid per litre)

| Carbon number of FFA | Solubility in water (g/l) |
|---|---|
| 2 | infinite |
| 4 | infinite |
| 6 | 9.7 |
| 8 | 0.7 |
| 10 | 0.15 |
| 12 | 0.055 |
| 14 | 0.02 |
| 16 | 0.007 |
| 18 | 0.003 |

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

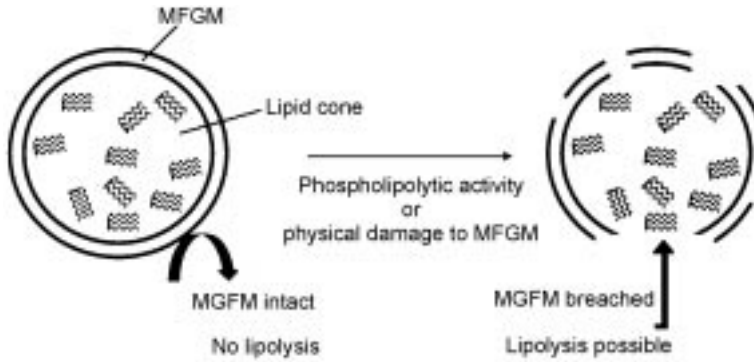



**Fig. 4.5** Correlation between phospholipolytic and lipolytic activity.

although for *P. fragi* lipase some preference has been reported for long chain fatty acids in the 1- and 3- positions of triglycerides (Fig. 4.4) to produce 2-monoglycerides (Lawrence, 1967). Another important characteristic of microbial lipases that differs from LPL is their extreme heat-tolerance, retaining significant activity even after UHT processing and production of milk powders (Chen *et al.*, 2003; Griffiths *et al.*, 1981) with *D*-values of 1410 s, 438 s and 120 s at 100ºC, 120ºC and 140ºC in skim milk medium, respectively, and a *z*-value of 37ºC in nutrient broth for *P. fluorescens* strain SIK W1 (Andersson *et al.*, 1979). Microbial lipases also appear not to be hindered by the MFGM (Christen *et al.*, 1986; Fitz-Gerald and Deeth, 1983), which can most likely be explained by the action of other enzymes such as phospholipases (Mabbit, 1981) (Fig. 4.5). Phospholipases, especially types C or lecithinase, which hydrolyses phosphatidylcholine in the MFGM, are produced by many types of bacteria including *Pseudomonas*, *Bacillus* and *Clostridium* (Cousin, 1982). These extracellular phospholipases are able to withstand various heat treatments (even UHT treatment) of milk (Deeth and Fitz-Gerald, 1983; Griffiths, 1983).

IP Address: 129.132.208.100

### 4.3.3 Chemical deterioration

Given the relatively short shelf-life of pasteurised milk, UHT and sterilised milk would theoretically be the most likely to be affected by light oxidation. However, most UHT milk is packaged in cartons that incorporate a layer of aluminium, which protects it during storage from light as well as $O_2$ penetration. As UHT milk has a shelf-life of several months, it is important to prevent both light transmission and oxygen permeation by choosing packaging material with barriers to light and oxygen.

Flavour changes in UHT milk during storage can be categorised by two stages. The flavours of UHT milk during the first stage can be described as cooked and sulphurous (Hansen *et al.*, 1974; Hansen and Swartzel, 1982; Mehta, 1980) and are due to free sulfhydryl (–SH) groups and volatile sulfides liberated via heat denaturation of the protein $\beta$-lactoglobulin. The –SH groups of heated

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

milk disappear after several days of storage, probably through oxidation (Lyster, 1964; Thomas *et al.*, 1975). The second stage of UHT milk flavour is mainly due to lipid oxidation, which produces oxidised and stale off-flavours. Another major defect associated with UHT milk is denaturation of whey proteins due to severe heat treatment, particularly $\beta$-lactoglobulin, which interacts with $\kappa$-casein, resulting in gelation and sedimentation during storage (Burton, 1969; Haque and Kinsella, 1988; Kirk *et al.*, 1968). The Maillard reaction is a chemical reaction between an amino acid and a reducing sugar, usually requiring heat. Lactuloselysine and fructoselysine are Maillard products formed in considerable amounts during storage of UHT milk at 30–37ºC for 6 months to 3 years (Moller *et al.*, 1977).

During storage for 90 days no loss was observed of vitamin A, carotene, vitamin E, thiamine, riboflavin, pantothenic acid, biotin and nicotinic acid (Ford *et al.*, 1968). Little or no loss of vitamin B6 or vitamin B12 was observed on processing, but up to 50% was lost during storage. All the dehydroascorbic acid and about 20% of the ascorbic acid was lost on processing. Stability of ascorbic acid in milk during storage diminishes with increased levels of dissolved oxygen, while folic acid is stabilised by the presence of ascorbic acid (Ford *et al.*, 1968).

### 4.3.4 Factors influencing spoilage

*Highly heat-resistant spore formers: role of farm and dairy*

IP Address: 129.132.208.100

A molecular typing study has shown that only a few clones, with a predominance of the HRS clone, have been responsible in the mid-1990s, and still are occasionally, for the contamination of UHT and sterilised milk and milk products due to the production of highly heat-resistant spores (Guillaume-Gentil *et al.*, 2002). Probably, the spread of the HRS clone has been caused by reprocessing and circulation of contaminated milk within and between UHT production units. Reprocessing of one contaminated package can contaminate a considerable fraction of the whole day's production at a level of one spore per litre, which is regarded as the common contamination level. There are indications that hydrogen peroxide used at sublethal sanitising conditions may have induced a higher heat resistance of *B. sporothermodurans* spores (Scheldeman *et al.*, 2006). The spread to other continents may be explained by the use of contaminated milk powder to reconstitute milk for UHT processing. Occasionally, a new genetic type, as exemplified by a small German UHT clone (Guillaume-Gentil *et al.*, 2002), is introduced in a UHT plant, probably via raw milk. *B. sporothermodurans* has been isolated from raw farm milk, although at a very low frequency and contamination level (Scheldeman *et al.*, 2006). At the dairy farm level feed concentrate is the most probable primary source, with the other positive farm samples probably resulting from contamination cycles on the farm (de Silva *et al.*, 1998; Scheldeman *et al.*, 2002; Vaerewijck *et al.*, 2001; Zhang *et al.*, 2002). As already explained in Section 4.3.2, contamination of raw milk at the farm is possible via the feed through faecal excretion. *B.*

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

*sporothermodurans* has been claimed to be isolated from cattle faeces (Wu *et al.*, 2007). Concentrate often contains tropical ingredients (e.g. coconut, citrus pulp, manioc, cacao), and it could thus be speculated that *B. sporothermodurans* has a (sub)tropical origin.

*The dairy cold chain*

The European legislation (Commission Regulation (EC), 2006) requires that fresh raw milk must be cooled immediately to not more than 8ºC in the case of daily collection, or not more than 6ºC if collection is not daily. During transport the cold chain must be maintained and, on arrival at the establishment of destination, the temperature of the milk must not be more than 10ºC. At the processing facility, the milk must be rapidly cooled to maximally 6ºC (unless processing starts immediately or within 4 hours after receipt). In Belgium, a coordinate set of strict guidelines called IKM (Integrale Kwaliteitszorg Melk – Dairy Quality Assurance Scheme, 2009) is imposed to ensure the quality of the entire Belgian production of unprocessed milk. According to these guidelines, within 2 hours after milking, milk temperature should have dropped to a maximum of 4ºC or less, keeping the milk temperature between two milking episodes between 4ºC (maximum temperature) and 1ºC (minimum temperature). Two-day-old or three-day-old cooled milk is then collected by the dairy company in a thermally isolated milk tanker. The milk is collected at approximately 4ºC and is kept at a temperature as low as possible (not exceeding 10ºC). At the dairy factory, the milk is ideally stored at a temperature of approximately 4ºC prior to processing, but higher temperatures up to 10ºC can occur. Since storage of raw milk for 24 hours or longer at the dairy factory before heat treatment may occur, several pre-processing methods (e.g. thermisation) can be applied to prevent deterioration of raw milk at the dairy factory prior to processing. Despite these efforts to keep total bacterial count as low as possible, cold storage favours the outgrowth of the psychrotrophic microbiota already present in raw milk (Fig. 4.6). The psychrotrophic microbiota is dominated by *Pseudomonas* that is able to outgrow other genera such as *Aeromonas*, *Listeria*, *Staphylococcus*, *Enterococcus* and the family of Enterobacteriaceae (Lafarge *et al.*, 2004).

The milking equipment, storage tanks and milk tankers are considered the major contamination source for psychrotrophic bacteria (Cousin, 1982). The use of untreated water supplies for the final rinse of the milking equipment may contribute to the contamination of raw milk with psychrotrophic micro-organisms. A likely reservoir from which contamination of these water supplies originate is the soil. Even though proper cleaning of the equipment effectively reduces contamination from these sources, the rubber materials used to connect different pipelines are quite susceptible to deterioration caused by a combination of high cleaning temperatures and strongly oxidising products in the dis-infectants. The resultant microscopic cracks and cuts form an ideal attachment place for the formation of biofilms (Morse *et al.*, 1968), which contain high numbers of bacteria and are highly resistant to chemical sanitisers (Simões *et al.*, 2009).

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

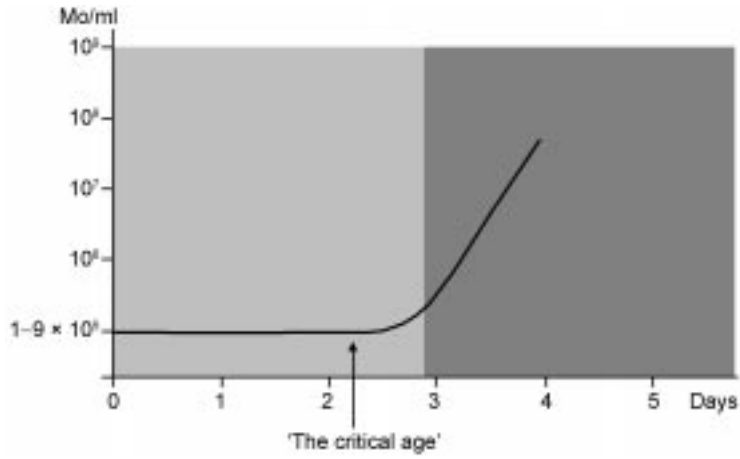



**Fig. 4.6** Psychrotrophic bacterial growth at +4ºC in raw milk. Taken from *Dairy Processing Handbook* (Bylund, 1995).

*Factors influencing stability of UHT milk*

Gelation occurs in different UHT milks after different periods of storage. The reason for this variability can be largely explained in terms of several influencing factors such as mode and severity of heat treatment, proteolysis, milk production factors, microbiological quality of raw milk, storage temperature and fat content (Datta and Deeth, 2001; Grufferty and Fox, 1988; Manji *et al.*, 1986; Manji and Kakuda, 1988). For equivalent bactericidal effects, milk sterilised by direct heating methods tends to gel sooner during storage than milk treated by indirect methods. The greater stability of indirectly heated milks is attributable to the higher heat load or increased severity of heating in indirect systems (Manji *et al.*, 1986; Manji and Kakuda, 1988). Milk production factors, such as the age of the cows, stage of lactation and mastitis, may influence the gelation of UHT treated milk. However, these three factors can be reduced to an increase in plasmin levels (Datta and Deeth, 2001). Seasonal variation in the composition of milk may, on the other hand, indirectly affect the gelation behaviour of UHT treated milk, since summer milk has been reported to give a more stable UHT product than winter milk (Ellerton and Pearce, 1964; Finley *et al.*, 1968; Graf and Bauer, 1976). Also the microbiological contamination of the raw milk is an important factor, as heat-resistant proteases produced by psychrotrophic bacteria cause the most serious gelation problems. In general, gelation occurs more readily at room temperature than at low (~4ºC) and high (~35–40ºC) storage temperatures (Kocak and Zadow, 1985). Finally, UHT processed skim milk is more susceptible to gelation than UHT whole milk due to an enhanced action of plasmin and bacterial proteases in skim milk compared to whole milk (Lopez-Fandino *et al.*, 1993).

After processing at the dairy factory, milk can become recontaminated with microorganisms when exposed to contaminated air, mainly during the filling

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

step (Eneroth *et al.*, 1998). The homogeniser can also be a source of recontamination when it is placed after the UHT installation, as is the case with direct UHT treatment. Since heat treatment affects the growth rate of spoilage microbiota by destroying the inhibitor mechanisms that are naturally present in milk (e.g., the lactoperoxidase system) (Wolfson and Sumner, 1993), post-processing contaminants may be able to grow more rapidly in heat-treated milk than in the raw product.

Finally, continuing breeding and management systems that focus solely on increasing milk and milk fat yield will result in a steady dilution of fat-soluble antioxidants (Ellis *et al.*, 2007; Jensen *et al.*, 1999; Nozière *et al.*, 2006; Slots *et al.*, 2009). Similarly, the production of low-fat milk will also reduce the content of fat-soluble antioxidants (Kaushik *et al.*, 2001).

### 4.3.5 Current methods to prevent spoilage

Current EU regulation for raw milk quality at the moment of collection requires that total bacterial count does not exceed $10^5$ cfu/ml (Commission Regulation (EC), 2006). Ideally, the ratio of total bacterial versus psychrotrophic bacterial count is 6 : 1. Changes in this ratio are regarded as a frequent cause of unexplained problems in milk processing (Cempirkova, 2002). Four factors are important in the pursuit of a better microbiological quality of the raw milk throughout the dairy chain: (1) the amount of bacteria that are initially present in the raw milk, since a high initial contamination results in a rapid outgrowth of psychrotrophic bacteria in raw milk (Thomas, 1966); (2) the type of bacteria; (3) storage temperature; and (4) storage time. Good hygienic practices in all aspects of milk handling, strict maintenance of refrigeration at 4ºC or lower, minimisation of the storage time of raw milk, combined with a suitable method to remove or kill microorganisms and followed up by an effective HACCP system, are therefore important parameters of primary concern in the dairy sector. Care should be taken that intensive washing of milking equipment and udder cleaning do not result in raw milk containing a majority of spoilage microorganisms such as *Pseudomonas* spp. and coliforms. This can be prevented by using hot water (>50ºC) and a sanitiser and by thorough washing and drying of the teats followed by wiping with disinfectant-impregnated towels or dipping solutions. The use of a lower raw milk storage temperature should not be combined with a prolonged storage time, as these conditions prior to processing create a selective advantage for psychrotrophic populations that can start to increase after a storage time of no more than 24 h at 4ºC (Lafarge *et al.*, 2004).

By subjecting raw materials to drastic heat treatments, even extremely heat-resistant *B. sporothermodurans* spores would be rendered inactive. Unfortunately, severe heat treatments are not well tolerated by milk because of negative organoleptic and nutritional effects, e.g. a considerable increase in lactulose content exceeding 400 mg/kg. Therefore, a heat treatment process of milk has to be designed to ensure a safe product with acceptable organoleptic and nutritional properties. To evaluate the safety of commonly applied heat

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

treatments in the dairy industry, it is important to know the heat resistance of spores. The current official methods to calculate sterility of thermally processed foods are based on the assumption that microbial heat inactivation follows a first-order kinetics. Hence, the decimal reduction time or '*D*-value', which is the time needed to reduce the size of the treated population by a factor of 10, can be used as a measure of the spore's heat resistance at the corresponding temperature. It is also assumed that the temperature dependence of *D* is log-linear, which produces the '*z*-value', i.e. the temperature interval at which *D* will decrease (or increase) by a factor of 10. Reference values for heat resistance of *B. sporothermodurans* spores are $D_{140°C}$ values varying between 3.4 and 7.9 s and a *z*-value between 13.1 and 14ºC (Huemer *et al.*, 1998; Scheldeman *et al.*, 2006). Compared with *Geobacillus* (previously *Bacillus*) *stearothermophilus* spores, this means that *B. sporothermodurans* spores are equally or even less heat resistant than *G. stearothermophilus* at sterilisation temperature (121ºC), but are exceptionally heat resistant at UHT temperatures (Fig. 4.7). This means that a 6 log reduction of HRS spores can only be achieved at a higher heating temperature of approximately 148ºC for 5 s, which would be sufficient to have a spoilage rate of 1 in $10^4$–$10^6$ one-litre packages assuming an initial spore load between 1 and 100 spores per litre of raw milk. Alternatively, bactofugation of

IP Address: 129.132.208.100

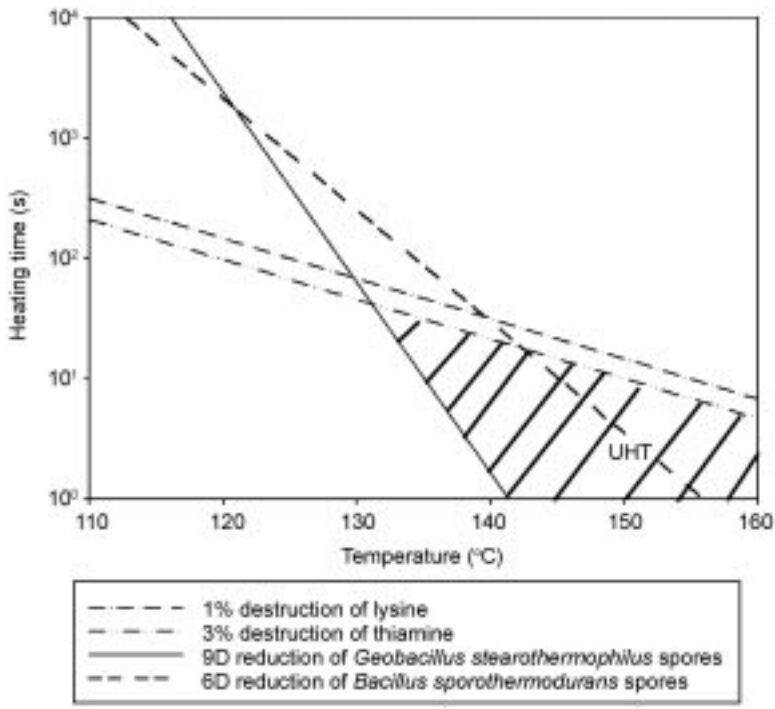



**Fig. 4.7** Heat inactivation and denaturation curves in the UHT region for thermophilic and *B. sporothermodurans* spores and for a milk vitamin and amino acid, respectively. The hatched area indicates the UHT region practically used in the dairy industry.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

raw milk (1–2 log spore reduction) can be combined with a lower heating regime of the bactofuged milk.

### 4.3.6 Emerging methods to prevent spoilage and deterioration

A newly developed and patented heating technique for milk is the innovative steam injection (ISI) heating system, which enables fast heating (shorter than 0.2 s holding time) and high temperatures (150–180ºC). With this ISI-heating technology, including pre- or post-heating at 80ºC, it is possible to reduce the amount of active plasmin to below 1% of the initial concentration and to achieve a 6 log reduction of *B. sporothermodurans* spores with less product degradation and improved taste characteristics compared to conventional UHT products (van Asselt *et al.*, 2008).

The demand for food with high nutrient value and high sensory quality has increased. According to Simopoulos (2002), consumers should lower their intake of *n*-6 fatty acids and increase their intake of *n*-3 fatty acids. Since the fatty acid distribution of milk fat is dependent on dietary composition, changing the feeding for the dairy cow will modify the composition of the fatty acids in milk, especially the polyunsaturated fatty acids (Dewhurst *et al.*, 2003). An increased proportion of unsaturated fat in milk may increase the oxidative susceptibility of milk, and to maintain a high quality, the concentration of antioxidants should therefore also be elevated. In milk, the concentration of $\alpha$-tocopherol and carotenoids as antioxidants is believed to be important for the oxidative stability, since they scavenge lipid peroxy radicals and quench singlet oxygen (Frankel, 2007). However, there are contradictory results in this field because earlier work shows prooxidative behaviour of $\alpha$-tocopherol, when no co-antioxidant, such as coenzyme Q, is present (Thomas *et al.*, 1996) or when a high concentration of unsaturated fat and $\alpha$-tocopherol is present in milk (Slots *et al.*, 2007).

Nowadays, all-rac-$\alpha$-tocopheryl acetate is the international standard vitamin E compound, which has been used as a feed supplement for years in order to improve the oxidative stability of milk (Blatt *et al.*, 2004). However, previous studies have indicated that supplementation with high concentrations (up to 10,000 IU/day) of all-rac-$\alpha$-tocopheryl acetate gave rise to only a modest increase in $\alpha$-tocopherol in the milk (Atwal *et al.*, 1990; Charmley *et al.*, 1993; Focant *et al.*, 1998; Kay *et al.*, 2005; St-Laurent *et al.*, 1990; Weiss and Wyatt, 2003). It is known that all-rac-$\alpha$-tocopheryl acetate consists of an equimolar mixture of all eight possible stereoisomers (RRR, RRS, RSS, RSR, SRR, SSR, SRS and SSS), each of them having its own biological activity (Fig. 4.8) (Jensen and Lauridsen, 2007). Several studies have shown that cows preferentially transfer the natural RRR-$\alpha$-tocopherol into the milk and blood system. In addition, the three synthetic 2R-stereoisomers were also excreted into the milk in limited amounts, while the synthetic 2S-stereoisomers were not detected in the milk (Meglia *et al.*, 2006; Slots *et al.*, 2007). Accordingly, Slots *et al.* (2007) suggested that supplementation of the feed with all-rac-$\alpha$-tocopheryl acetate is

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

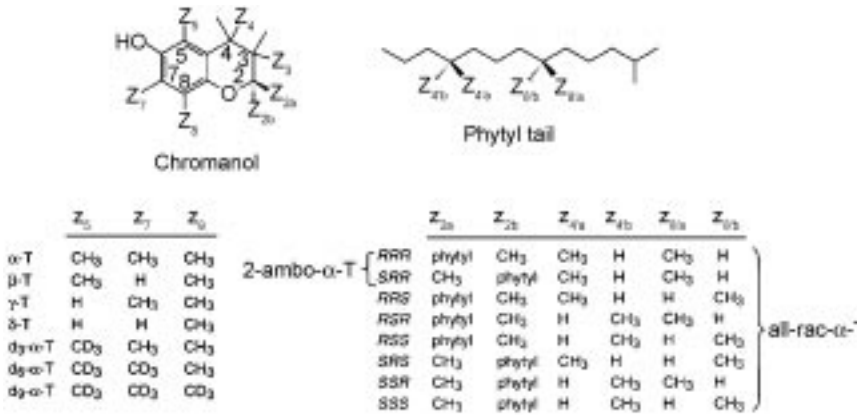



**Fig. 4.8** The different forms of vitamin E. T in the figure represents tocopherol. Taken from Burton *et al.* (1998).

an ineffective way to increase the content of $\alpha$-tocopherol in milk. Most concentrate feeds eaten by cows are very low in carotenoids, although high concentrations of retinyl palmitate (vitamin A) are often supplemented. Carotenoids detected in milk usually originate from roughage (Nozière *et al.*, 2006). In addition to concentrate supplements, vitamin E and $\beta$-carotene content of milk can also be increased by changing the feeding regime of cows. Havemose *et al.* (2004) observed significantly higher amounts of fat-soluble antioxidants, $\alpha$-tocopherol and $\beta$-carotene, in milk from cows fed high amounts of grass silage compared with milk from cows fed high amounts of maize silage. When comparing organic milk with conventional milk, higher concentrations of fat-soluble antioxidants could be detected in organic milk (Bergamo *et al.*, 2003; Butler *et al.*, 2008; Ellis *et al.*, 2007; Slots *et al.*, 2008). An earlier study by Schingoethe *et al.* (1978) has shown that $\alpha$-tocopherol in the feed is progressively oxidised during storage of the feed, leading to inadequate $\alpha$-tocopherol intake by the cows, especially during winter and spring months.

As an alternative to the direct addition into UHT milk of antioxidants such as ascorbic acid (Jeon *et al.*, 1976), butylated hydroxyl anisole (BHA) (Wadsworth and Basette, 1985) and bioflavonoids (Morgan *et al.*, 1971), active packaging incorporating oxygen scavengers can be used. For liquid foods it is preferable to have the scavenger incorporated into the package itself to allow a greater contact surface with the product. Perkins *et al.* (2005) showed that incorporation of an oxygen-scavenging film into a UHT milk package resulted in the product having significantly ($p < 0.05$) lower dissolved oxygen levels, 23–28% below the control, during room-temperature storage for 14 weeks. Corresponding with this, significant reductions in stale-flavour volatiles were obtained. These results suggest that use of an active packaging system may be successful in controlling staling in UHT milk.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

## 4.4 Future trends

Consumers are looking for more natural products with increased freshness, while distributors and retailers demand products with an extended shelf-life. These two demands appear conflicting, but the entire dairy sector will have to look further for agricultural practices, formulations, processes and packaging systems which meet and keep these demands. Further improvements must come from risk assessment and HACCP strategies, which target not only pathogens but also spoilers (Koutsoumanis, 2009). For this purpose, it is important that the dairy industry adopts more rapid and specific methods to detect, enumerate and identify specific spoilage microorganisms and chemical defects to replace the classical microbiological and chemical techniques. These techniques can be based on bacterial genomics (Marco and Wells-Bennik, 2008) or metabolomics (e.g. gas-sensor array technology, Fourier transform infrared spectroscopy) (Haugen *et al.*, 2006; Nicolaou and Goodacre, 2008). This would allow more accurate determinations of shelf-life and the implementation of directed control measures.

An important specific preventive measure is to target the mechanisms of biofilm formation such as interference with bacterial signalling molecules (quorum sensing), use of bacteriophages as control agents before or with chemical biocides, molecular brushes (e.g. polyethylene glycol, PEG) to block microbial attachment, and active coatings. Recently, the use of a *P. fluorescens* bacteriophage was demonstrated for the removal of biofilms (Sillankorva *et al.*, 2008). Biosensor technologies may provide further solutions to the food industry to monitor biofilms in the future (Brooks and Flint, 2008). Also further insights into the mechanisms of resistance of spores to biocides can lead to potential combinations of sporicidal agents or combinations of potentiators with sporicides. An interesting research topic would be to learn more of the involvement of quorum sensing signalling systems in the spoilage of foods; this knowledge could lead to potential means to prevent or delay food spoilage by the disruption or control of cell-to-cell communication (quorum quenching) (Ammor *et al.*, 2008).

IP Address: 129.132.208.100

## 4.5 Sources of further information and advice

Websites:

- International Dairy Federation, http://www.fil-idf.org: major professional body.
- Tetra Pak, http://www.tetrapak.com: major provider of dairy technology and packaging.
- Dairy Quality Assurance Scheme (DQA) in Belgium, http://www.ikm.be/home_en.phtml.

Book chapters dealing with aerobic spore formers and their spoilage defects in milk (for details see Section 4.6):

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

- Heyndrickx M (2010), 'Dispersal of aerobic endospore-forming bacteria from soil and agricultural activities to food and feed'
- Heyndrickx M and Scheldeman P (2002), 'Bacilli associated with spoilage in dairy products and other food'.

Key books on general items related to milk spoilage:

- *Encyclopedia of Dairy Sciences* (2003). Editor-in-chief: Roginski H. Academic Press, London.
- *Essentials of the Microbiology of Foods: a Textbook for Advanced Studies* (1995). Editors: Mossel D A A *et al.* John Wiley & Sons, Chichester.

Key books on specific items related to milk spoilage:

- *Control of Foodborne Microorganisms* (2002). Editors: Juneja V K and Sofos J N. Marcel Dekker, New York.
- *Enzymes of Psychrotrophs in Raw Food* (1989). Editor: McKellar R C. CRC Press, Boca Raton, FL.
- *Dairy Chemistry and Biochemistry* (1998). Editors: Fox P F and McSweeney P L H. Blackie Academic and Professional, London.
- *A Colour Atlas of Food Quality Control* (1986). Editors: Sutherland J P *et al.* Wolfe Publishing, London.

Key books on technology and systems to prevent spoilage:

- *Introduction to Food Engineering* (2009). Editors: Singh R P and Heldman D R. Elsevier, Amsterdam.
- *Food Industry Briefing Series: Shelf Life* (2002). Editor: Man D. Blackwell Science, Oxford.
- *Dairy Processing and Quality Assurance* (2008). Editors: Chandan R C *et al.* Wiley-Blackwell, Ames, IA.
- *Biofilms II: Process Analysis and Applications* (2000). Editor: Bryers J D. Wiley-Liss, New York.
- *Applying HACCP-based Quality Risk Management on Dairy Farms* (2008). Editors: Noordhuizen J *et al.* Wageningen Academic Publishers, Wageningen, the Netherlands.
- *Dairy Processing Handbook* (2003). Publisher: Tetra Pak Processing Systems AB, Lund, Sweden.

IP Address: 129.132.208.100

## 4.6 References

ADLER-NISSEN J (1986), *Enzymatic Hydrolysis of Food Proteins*, London, Elsevier Applied Science Publishers.

ALLEN C and PARKS O W (1979), 'Photodegradation of riboflavin in milks exposed to fluorescent light', *J Dairy Sci*, 62, 1377–1379.

ALLEN J C and JOSEPH G (1985), 'Deterioration of pasteurized milk on storage', *J Dairy Res*, 52, 469–487.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

AMMOR M S, MICHAELIDIS C and NYCHAS G J E (2008), 'Insights into the role of quorum sensing in food spoilage', *J Food Prot*, 71, 1510–1525.

ANDERSON A and RÖNNER U (1998), 'Adhesion and removal of dormant, heat-activated and germinated spores of three strains of *Bacillus cereus*', *Biofouling*, 13, 51–67.

ANDERSSON R E, HEDLUND C B and JONSSON U (1979), 'Thermal inactivation of a heat-resistant lipase produced by the psychrotrophic bacterium *Pseudomonas fluorescens*', *J Dairy Sci*, 62, 361–367.

ANDREW A T and ALICHANIDIS E (1983), 'Proteolysis of caseins and the proteose peptone fraction of bovine milk', *J Dairy Res*, 50, 275–290.

ARPIGNY J L and JAEGER K E (1999), 'Bacterial lipolytic enzymes: classification and properties', *Biochem J*, 343, 177–183.

ATWAL A S, HIDIROGLOU M, KRAMER J K G and BINNS M R (1990), 'Effects of feeding $\alpha$-tocopherol and calcium salts of fatty acids on vitamin E and fatty acid composition of cow's milk', *J Dairy Sci*, 73, 2832–2841.

BARBANO D M, MA Y and SANTOS M V (2006), 'Influence of raw milk quality on fluid milk shelf-life', *J Dairy Sci*, 89, E15–E19.

BARTOSZEWIEZ M, HANSEN B M and SWIECICKA I (2008), 'The members of the *Bacillus cereus* group are commonly present contaminants of fresh and heat-treated milk', *Food Microbiol*, 25, 588–596.

BASTIAN E D and BROWN R J (1996), 'Plasmin in milk and dairy products: an update', *Int Dairy J*, 6, 435–457.

BERGAMO P, FEDELE E, IANNIBELLI L and MARZILLO G (2003), 'Fat-soluble vitamin contents and fatty acid composition in organic and conventional Italian dairy products', *Food Chem*, 82, 625–631.

BLATT D H, PRYOR W A, MATA J E and RODRIGUEZ-PROTEAU R (2004), 'Re-evaluation of the relative potency of synthetic and natural $\alpha$-tocopherol: experimental and clinical observations', *J Nutr Biochem*, 15, 380–395.

BOSSET J O, GALLMANN P U and SIEBER R (1993), 'Influence of light transmittance of packing materials on the shelf-life of milk and dairy-products – a review', *Lait*, 73, 3–49.

BOSSIS E, LEMANCEAU P, LATOUR X and GARDAN L (2000), 'The taxonomy of *Pseudomonas fluorescens* and *Pseudomonas putida*: current status and need for revision', *Agronomie*, 20, 51–63.

BRAUN P, FEHLHABER K, KLUG C and KOPP K (1999), 'Investigations into the activity of enzymes produced by spoilage-causing bacteria: a possible basis for improved shelf-life estimation', *Food Microbiol*, 16, 531–540.

BROOKS J D and FLINT S H (2008), 'Biofilms in the food industry: problems and potential solutions', *Int J Food Sci Technol*, 43, 2163–2176.

BRYERS J D (2000), *Biofilms II: Process Analysis and Applications*, New York, Wiley-Liss Inc.

BURGER M, WOODS R G, MCCARTHY C and BEACHAM I R (2000), 'Temperature regulation of protease in *Pseudomonas fluorescens* LS107d2 by an ECF sigma factor and a transmembrane activator', *Microbiol*, 146, 3149–3155.

BURTON G W, TRABER M G, ACUFF R V, WALTERS D N, KAYDEN H, HUGHES L and INGOLD K U (1998), 'Human plasma and tissue $\alpha$-tocopherol concentrations in response to supplementation with deuterated natural and synthetic vitamin E', *Am J Clin Nutr*, 67, 669–684.

BURTON H (1969), 'Ultra-high-temperature processed milk: a review', *Dairy Sci Abstr*, 31, 287–297.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

BURTON H (1988), *Ultra-high-temperature Processing of Milk and Milk Products*, London, Elsevier Applied Science Publishers.

BUTLER G, NIELSEN J H, SLOTS T, SEAL C, EYRE M D, SANDERSON R and LEIFERT C (2008), 'Fatty acid and fat-soluble antioxidant concentrations in milk from high- and low-input conventional and organic systems: seasonal variation', *J Sci Food Agric*, 88, 1431–1441.

BYLUND G (1995), *Dairy Processing Handbook*, Lund, Sweden, Tetra Pak Processing Systems AB.

CARLIN F, FRICKER M, PIELAAT A, HEISTERKAMP S, SHAHEEN R, SALKINOJA-SALONEN M, SVENSSON B, NGUEN-THE C and EHLING-SCHULZ M (2006), 'Emetic toxin-producing strains of *Bacillus cereus* show distinct characteristics within the *Bacillus cereus* group', *Int J Food Microbiol*, 109, 132–138.

CEMPIRKOVA R (2002), 'Psychrotrophic vs. total bacterial counts in bulk milk samples', *Vet Med Czech*, 47, 227–233.

CHABEAUD P, DE GROOT A, BITTER W, TOMMASSEN J, HEULIN T and ACHOUACK W (2001), 'Phase variable expression of an operon encoding extracellular alkaline protease, a serine protease homolog, and lipase in *Pseudomonas brassicacearum*', *J Bacteriol*, 183, 2117–2120.

CHANDAN R C, KILARA A and SHAH N P (2008), *Dairy Processing and Quality Assurance*, Ames, IA, Wiley-Blackwell.

CHAPMAN K W, AWLESS H T and BOOR K J (2009), 'Quantitative descriptive analysis and principal component analysis for sensory characterization of ultrapasteurized milk', *J Dairy Sci*, 84, 12–20.

CHARMLEY E, NICHOLSON J W G and ZEE J A (1993), 'Effect of supplemental vitamin E and selenium in the diet on vitamin E and selenium levels and control of oxidized flavor in mik from Holstein cows', *Can J Animal Sci*, 73, 453–457.

CHEN L, DANIEL R M and COOLBEAR T (2003), 'Detection and impact of protease and lipase activities in milk and milkpowders', *Int Dairy J*, 7, 255–275.

CHESSA J P, PETRESCU I, BENTAHIR M, VAN BEEUMEN J and GERDAY C (2000), 'Purification, physico-chemical characterization and sequence of a heat-labile alkaline metalloprotease isolated from a psychrophilic *Pseudomonas* species', *Biochim Biophys Acta*, 1479, 265–274.

CHING-HSING L and MCCALLUS D E (1998), 'Biochemical and genetic characterization of an extracellular protease from *Pseudomonas fluorescens* CY091', *Appl Environ Microbiol*, 64, 914–921.

CHRISTEN G L, WANG W C and REN T J (1986), 'Comparison of the heat resistance of bacterial lipases and proteases and the effect on ultra-high temperature milk quality', *J Dairy Sci*, 69, 2769–2778.

COMMISSION DIRECTIVE (1992), '92/46/EEC of 16 June 1992 laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products', *OJ*, L268, 14 September 1992, 1–32.

COMMISSION REGULATION (EC) (2006), 'No 1662/2006 of 6 November 2006 amending Regulation (EC) No 853/2004 of the European Parliament and of the Council laying down specific rules for food of animal origin', *OJ*, L320, 18 November 2006, 1–10.

CORRADINI C and PECCHINI G (1981), 'Effects of proteinases of different UHT ultra high temperature treatment', *Neth Milk Dairy J*, 35, 393–395.

COUSIN M A (1982), 'Presence and activity of psychrotrophic microorganisms in milk and dairy products: a review', *J Food Prot*, 45, 172–207.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

CRUDDEN A, FOX P F and KELLY A L (2005), 'Factors affecting the hydrolytic action of plasmin in milk', *Int Dairy J*, 15, 305–313.

CYBERLIPID CENTER (2009), available from http://www.cyberlipid.org/index.htm (accessed 27 July 2009).

DAIRY MANAGEMENT, INC. (2001), 'Achieving extended shelf-life in fluid milk: creating hurdles for spoilage factors', *Innovations in Dairy Industry Technology Review*, 1–8.

DAIRY QUALITY ASSURANCE SCHEME (DQA) (2009), available from http://www.ikm.be/home_en.phtml (accessed 25 August 2009).

DATTA N and DEETH H C (2001), 'Age gelation of UHT milk: a review', *Trans IChemE*, 79, 197–210.

DATTA N and DEETH H C (2003), 'Diagnosing the cause in proteolysis in UHT milk', *Lwt – Food Sci Technol*, 36, 173–182.

DE JONGHE V, COOREVITS A, DE BLOCK J, VAN COILLIE E, GRIJSPEERDT K, HERMAN L, DE VOS P and HEYNDRICKX M (2010), 'Toxicity and spoilage potential of aerobic spore-formers isolated from raw milk', *Int J Food Microbiol*, 136, 318–325.

DE SILVA S, PETTERSON B, DE MURO M A and PRIEST F G (1998), 'A DNA probe for the detection and identification of *Bacillus sporothermodurans* using the 16S-23S rDNA spacer region and phylogenetic analysis of some field isolates of *Bacillus* which form highly heat resistant spores', *Syst Appl Microbiol*, 21, 398–407.

DE VRIES Y P (2006), '*Bacilus cereus* spore formation, structure and germination', PhD thesis, Wageningen University, Wageningen, the Netherlands.

DEETH H C (2006), 'Lipoprotein lipase and lipolysis in milk', *Int Dairy J*, 16, 555–562.

DEETH H C and FITZ-GERALD C H (1983), 'Lipolytic enzymes and hydrolytic rancidity in milk and milk products', in Fox P F, *Developments in Dairy Chemistry. Part II*, London, Applied Science Publishers, 195–239.

DEETH H C, KHUSNIATI T, DATTA N and WALLACE R B (2002), 'Spoilage patterns of skim and whole milks', *J Dairy Res*, 69, 227–241.

DEWHURST R J, SCOLLAN N D, LEE M R F, OUGHAM H J and HUMPHREYS M O (2003), 'Forage breeding and management to increase the beneficial fatty acid content of ruminant products', *Proc Nutr Soc*, 62, 329–336.

DIAZ O, GOULDSWORTHY A M and LEAVER J (1996), 'Identification of peptides released from casein micelles by limited trypsinolysis', *J Agric Food Chem*, 44, 2517–2522.

DIMICK P S (1973), 'Effect of fluorescent light on the flavor and selected nutrients of homogenized milk held in conventional containers', *J Milk Food Technol*, 36, 383–387.

DIMICK S and KILARA A (1983), 'Photooxidative changes in milk proteins and amino acids', *Kiel Milchwirtsch Forschungsber*, 35, 289–299.

DOGAN Z, WEIDENDORFER M, MÜLLERBACH-MERBACH M, LEMBKE F and HINRICHS J (2009), 'Inactivation kinetics of *Bacillus* spores in batch- and continuous heating systems', *Lwt – Food Sci Technol*, 42, 81–86.

DRIEHUIS F and OUDE ELFERINK S J (2000), 'The impact of the quality of silage on animal health and food safety: a review', *Vet Q*, 22, 212–216.

DUNKLEY W L and FRANKE A A (1967), 'Evaluating susceptibility of milk to oxidized flavor', *J Dairy Sci*, 50, 1–9.

DUONG F, LAZDUNSKI A, CAMI B and MURGIER M (1992), 'Sequence of a cluster of genes controlling synthesis and secretion of alkaline protease in *Pseudomonas aeruginosa*: relationships to other secretory pathways', *Gene*, 121, 47–54.

ELLERTON M E and PEARCE S J (1964), 'Some observations on the physical–chemical stability of sterile concentrated milks', *J Dairy Sci*, 47, 564–569.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

ELLIS K A, MONTEIRO A, INNOCENT G T, GROVE-WHITE D, CRIPPS P, MCLEAN W G, HOWARD C V and MIHM M (2007), 'Investigation of the vitamins A and E and $\beta$-carotene content in milk from UK organic and conventional dairy farms', *J Dairy Res*, 74, 484–491.

ENEROTH A, CHRISTIANSSON A, BRENDEHAUG J and MOLIN G (1998), 'Critical contamination sites in the production line of pasteurised milk, with reference to the psychrotrophic spoilage flora', *Int Dairy J*, 8, 829–834.

ERCOLINI D, RUSSO F, BLAIOTTA G, PEPE O, MAURIELLO G and VILLANI F (2007), 'Simultaneous detection of *Pseudomonas fragi*, *P. lundensis*, and *P. putida* from meat by use of a multiplex PCR assay targeting the *carA* gene', *Appl Environ Microbiol*, 73, 2354–2359.

FAILLE C, FONTAINE F and BENEZECH T (2001), 'Potential occurrence of adhering living *Bacillus* spores in milk product processing lines', *J Appl Microbiol*, 90, 892–900.

FAIRBAIRN D J and LAW B A (1986), 'Proteinases of psychrotrophic bacteria: their production, properties, effects and control', *J Dairy Res*, 53, 139–177.

FAJARDO-LIRA C, ORIA M, HAYES K D and NIELSEN S S (2000), 'Effect of psychrotrophic bacteria and of an isolated protease from *Pseudomonas fluorescens* M3/6 on the plasmin system of fresh milk', *J Dairy Sci*, 83, 2190–2199.

FANG W and SANDHOLM M (1995), 'Inhibition of protease activity in mastitic milk', *J Dairy Sci*, 62, 61–68.

FILLAUDEAU L, WINTERTON P, LEULIET J C, TISSIER J P, MAURY V, SEMET F, DEBREYNE P, BERTHOU M and CHOPARD F (2006), 'Heat treatment of whole milk by the direct Joule effect – experimental and numerical approaches to fouling mechanisms', *J Dairy Sci*, 89, 4475–4489.

FINLEY R D, WARREN H B and HARGROVE R E (1968), 'Storage stability of commercial milk', *J Milk Food Technol*, 31, 382–387.

FITZ-GERALD C H and DEETH H C (1983), 'Factors influencing lipolysis by skim milk cultures of some psychrotrophic micro-organisms', *Aust J Dairy Technol*, 38, 97–103.

FOCANT M, MIGNOLET E, MARIQUE M, CLABOTS F, BREYNE T, DALEMANS D and LARONDELLE Y (1998), 'The effect of vitamin E supplementation of cow diets containing rapeseed and linseed on the prevention of milk fat oxidation', *J Dairy Sci*, 81, 1095–1101.

FORD J E, PORTER J W G, THOMPSON S Y, TOOTHILL J and EDWARDS W J (1968), 'Effects of UHT-processing and of subsequent storage on vitamin content of milk', *J Dairy Res*, 36, 447–454.

FOX P F (1981), 'Proteinases in dairy technology', *Neth Milk Dairy J*, 35, 233–253.

FOX P F and KELLY A L (2006), 'Indigenous enzymes in milk: Overview and historical aspects – Part 1', *Int Dairy J*, 16, 500–516.

FOX P F and MCSWEENEY P L H (1998), *Dairy Chemistry and Biochemistry*, London, Blackie Academic & Professional.

FRANKEL E N (2007), *Antioxidants in Food and Biology. Facts and Fiction*, Bridgwater, UK, The Oily Press.

GOFF H D and GRIFFITHS M W (2006), 'Major advances in fresh milk and milk products: fluid milk products and frozen desserts', *J Dairy Sci*, 89, 1163–1173.

GRAF E and BAUER H (1976), *Milk and Milk Products. Food Emulsions*, New York, Marcel Dekker.

GRIFFITHS M W (1983), 'Synergistic effects of various lipases and phospholipase C on milk fat', *J Food Technol*, 18, 495–505.

GRIFFITHS M W, PHILLIPS J D and MUIR D D (1981), 'Thermostability of proteases and lipases from a number of species of psychrotrophic bacteria of dairy origin', *J Appl Bacteriol*, 50, 289–303.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

GRUFFERTY M B and FOX P F (1988), 'Milk alkaline proteinase', *J Dairy Res*, 55, 609–30.

GUILLAUME-GENTIL O, SCHELDEMAN P, MARUGG J, HERMAN L, JOOSTEN H and HEYNDRICKX M (2002), 'Genetic heterogeneity in *Bacillus sporothermodurans* as demonstrated by ribotyping and repetitive extragenic palindromic-PCR fingerprinting', *Appl Environ Microbiol*, 68, 4216–4224.

GUINEBRETIÈRE M H, THOMPSON F L, SOROKIN A, NORMAND P, DAWYNDT P, EHLING-SCHULZ M, SVENSSON B, SANCHIS V, NGUYEN-THE C, HEYNDRICKX M and DE VOS P (2008), 'Ecological diversification in the *Bacillus cereus* group', *Environ Microbiol*, 10, 851–865.

GUPTA R, GUPTA N and RATHI P (2004), 'Bacterial lipases: an overview of production, purification and biochemical properties', *Appl Microbiol Biotechnol*, 64, 763–781.

HAMMER P, LEMBKE F, SUHREN G and HEESCHEN W (1995), 'Characterization of a heat resistant mesophilic *Bacillus* species affecting quality of UHT-milk – a preliminary report', *Kiel Milchwirtsch Forschungsber*, 47, 303–311.

HANSEN A P and SWARTZEL K R (1982), 'Taste panel testing of UHT fluid dairy products', *J Food Quality*, 4, 203–216.

HANSEN A P, TURNER L G and JONES V A (1974), 'Effects of ultra high temperature steam injection on flavor acceptability of whole and fortified skim milk', *J Dairy Sci*, 57, 280–284.

HANSON M L, WENDORFF W L and HOUCK K B (2005), 'Effect of heat treatment of milk on activation of *Bacillus* spores', *J Food Prot*, 68, 1484–1486.

HAQUE Z and KINSELLA J E (1988), 'Interaction between heated kappa-casein and beta-lactoglobulin: predominance of hydrophobic interactions in the initial stages of complex formation', *J Dairy Res*, 55, 67–80.

HARDING F (1995), 'Processed milk', in Harding F, *Milk Quality*, Glasgow, Blackie Academic & Professional, 112–132.

HAUGEN J E, RUDI K, LANGSRUD S and BREDHOLT S (2006), 'Application of gas-sensor array technology for detection and monitoring of growth of spoilage bacteria in milk: a model study', *Anal Chim Acta*, 565, 10–16.

HAVEMOSE M S, WEISBJERG M R, BREDIE W L P and NIELSEN J H (2004), 'Influence of feeding different types of roughage on the oxidative stability of milk', *Int Dairy J*, 14, 563–570.

HEYNDRICKX M (2010), 'Dispersal of aerobic endospore-forming bacteria from soil and agricultural activities to food and feed', in Logan N A and De Vos P, *Aerobic Endospore-forming Soil Bacteria*, Springer Soil Biology Series, Berlin, Springer-Verlag (to be published).

HEYNDRICKX M and SCHELDEMAN P (2002), 'Bacilli associated with spoilage in dairy products and other food', in Berkeley R C W, Heyndrickx M, Logan N A and De Vos P, *Applications and Systematics of Bacillus and Relatives,* Oxford, Blackwell Science, 65–82.

HICKEY D K, KILCAWLEY K N, BERESFORD T P and WILKINSON M G (2007), 'Lipolysis in Cheddar cheese made from raw, thermized, and pasteurized milks', *J Dairy Sci*, 90, 47–56.

HORNSTRA L M, DE LEEUW P L A, MOEZELAAR R, WOLBERT E J, DE VRIES Y P, DE VOS W M and ABEE T (2007), 'Germination of *Bacillus cereus* spores adhered to stainless steel', *Int J Food Microbiol*, 116, 367–371.

HUEMER I A, KLIJN N, VOGELSANG H W J and LANGEVELD L P M (1998), 'Thermal death kinetics of spores of *Bacillus sporothermodurans* isolated from UHT milk', *Int Dairy J*, 8, 851–855.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

INTERNATIONAL DAIRY FEDERATION (IDF) (1991), 'Determination of free fatty acids in milk and milk products', *Bull Int Dairy Fed*, 265, 26–40.

IN T'VELD J H J H (1996), 'Microbial and biochemical spoilage of foods: an overview', *Int J Food Microbiol*, 33, 1–18.

JAEGER K E, RANSAC S, DIJKSTRA B W, COLSON C, VAN HEUVEL M and MISSET O (1994), 'Bacterial lipases', *FEMS Microbiol Rev*, 15, 29–63.

JENQ W, BASSETTE R and CRANG R W (1988), 'Effects of light and copper ions on volatile aldehydes of milk and milk fractions', *J Dairy Sci*, 71, 2366–2372.

JENSEN R G (1983), 'Detection and determination of lipase acylglycerol hydrolase activity from various sources', *Lipids*, 18, 650–657.

JENSEN S K and LAURIDSEN C (2007), '$\alpha$-Tocopherol stereoisomers', *Vitamins and Hormones*, 76, 281–308.

JENSEN S K, JOHANNSEN A K B and HERMANSEN J E (1999), 'Quantitative secretion and maximal secretion capacity of retinol, $\beta$-carotene and $\alpha$-tocopherol into cows' milk', *J Dairy Res*, 66, 511–522.

JEON I J, THOMAS E L and REINECCIUS G A (1976), 'Production of volatile flavor compounds in ultra-high-temperature processed milk during aseptic storage', *Abstr Papers Am Chem Soc*, 172, 157.

JUHAS M, EBERL L and TÜMMLER B (2005), 'Quorum sensing: the power of cooperation in the world of *Pseudomonas*', *Environ Microbiol*, 7, 459–471.

JUNEJA V K and SOFOS J N (2002), *Control of Foodborne Microorganisms*, New York, Marcel Dekker.

JUNG M Y, LEE K H and KIM S Y (1998), 'Retinyl palmitate isomers in skim milk during light storage as affected by ascorbic acid', *J Food Sci*, 63, 597–600.

KAUSHIK S, WANDER R, LEONARD S, GERMAN B and TRABER M G (2001), 'Removal of fat from cow's milk decreases the vitamin E contents of the resulting dairy products', *Lipids*, 36, 73–78.

KAWAI E, IDEI A, KUMURA H, SHIMAZAKI K, AKATSUKA H and OMORI K (1999), 'The ABC-exporter genes involved in the lipase secretion are clustered with the genes for lipase, alkaline protease, and serine protease homologues in *Pseudomonas fluorescens* no. 33', *Biochim Biophys Acta*, 1446, 800–802.

KAY J K, ROCHE J R, KOLVER E S, THOMSON N A and BAUMGARD L H (2005), 'A comparison between feeding systems (pasture and TMR) and the effect of vitamin E supplementation on plasma and milk fatty acid profiles in dairy cows', *J Dairy Res*, 72, 322–332.

KELLY A L and MCSWEENEY P L H (2003), 'Indigenous proteinases in milk', in Fox P F and McSweeney P L, *Advanced Dairy Chemistry, Volume A: Proteins,* New York, Kluwer Academic/Plenum Publishers, 495–521.

KIM H-J, TAMANOUE Y, JEOHN G-H, IWAMATSU A, YOKOTA A, KIM Y-T, TAKAHASHI T and TAKAHASHI K (1997), 'Purification and characterization of an extracellular metalloprotease from *Pseudomonas fluorescens*', *J Biochem*, 121, 82–88.

KING J M and MIN D B (1998), 'Riboflavin photosensitized singlet-oxygen oxidation of vitamin D', *J Food Sci*, 63, 31–34.

KIRK J A, HEDRICK T I and STINE C M (1968), 'Gas chromatographic study of flavour deterioration in high-temperature short-time fluid sterile milk', *J Dairy Sci*, 51, 492–497.

KLIJN N, HERMAN L, LANGEVELD M A, VAEREWIJCK M and WAEGENDORP A A (1997), 'Genotypical and phenotypical characterization of *Bacillus sporothermodurans* strains, surviving UHT sterilisation', *Int Dairy J*, 7, 421–428.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

KOCAK H R and ZADOW J G (1985), 'Age gelation of UHT whole milk as influenced by storage temperature', *Aust J Dairy Technol*, 40, 14–21.

KOHLMANN K L, NIELSEN S S, STEENSON L R and LADISCH M R (1991), 'Production of proteinases by psychrotrophic microorganisms', *J Dairy Sci*, 74, 3275–3283.

KOSARIC N, KITCHEN B, PANCHAL C J, SHEPPARD J D, KENNEDY K and SARAGANT A (1981), 'UHT milk: production, quality and economics', *CRC Crit Rev Food Sci Nutr*, 14, 153–199.

KOUTSOUMANIS K (2009), 'Modeling food spoilage in microbial risk assessment', *J Food Prot*, 72, 425–427.

KUMETA H, HOSHINO T, GODA T, OKAYAMA T, SHIMADA T, OHGIYA S, MATSUYAMA H and ISHIZAKI K (1999), 'Identification of the serralysin family isolated from the psychrotrophic bacterium, *Pseudomonas fluorescens* 114', *Biosci Biotechnol Biochem*, 63, 1165–1170.

LAFARGE V, OGIER J C, GIRARD V, MALADEN V, LEVEAU J Y, GRUSS A and DELACROIX-BUCHET A (2004), 'Raw cow milk bacterial population shifts attributable to refrigeration', *Appl Environ Microbiol*, 70, 5644–5650.

LARSEN H D and JORGENSEN K (1997), 'The occurence of *Bacillus cereus* in Danish pasteurized milk', *Int J Food Microbiol*, 34, 179–186.

LARSEN H D and JORGENSEN K (1999), 'Growth of *Bacillus cereus* in pasteurized milk products', *Int J Food Microbiol*, 46, 173–176.

LAW B A (1979), 'Reviews in the progress of dairy science: enzymes of psychrotrophic bacteria and their effects on milk and milk products', *J Dairy Res*, 46, 573–588.

LAWRENCE C (1967), 'Microbial lipases and related esterases. Part 11. Estimation of lipase activity. Characterization of lipases. Recent work concerning their effect on dairy products', *Dairy Sci Abstr*, 29, 59–70.

LECHNER S, MAYR R, FRANCIS K P, PRUSS B M, KAPLAN T, WIESSNER-GUNKEL E, STEWARTZ G S A B and SCHERER S (1998), '*Bacillus weihenstephanensis* sp. nov. is a new psychrotolerant species of the *Bacillus cereus* group', *Int J Syst Bacteriol*, 48, 1373–1382.

LEE K H, JUNG M Y and KIM S Y (1998), 'Effects of ascorbic acid on the light-induced riboflavin degradation and color changes in milks', *J Agric Food Chem*, 46, 407–410.

LEWIS M J (1994), 'Heat treatment of milk', in Robinson R K, *Modern Dairy Technology*, London, Chapman & Hall, 1–60.

LIAO C H and MCCALLUS D E (1998), 'Biochemical and genetic characterization of an extracellular protease from *Pseudomonas fluorescens* CY091', *Appl Environ Microbiol*, 64, 914–921.

LIU M, WANG H and GRIFFITHS M W (2007), 'Regulation of alkaline metalloprotease promotor by *N*-acyl homoserine lactone quorum sensing in *Pseudomonas fluorescens*', *J Appl Microbiol*, 103, 2174–2184.

LOPEZ-FANDINO R, OLANO A, CORZO N and RAMOS M (1993), 'Proteolysis during storage of UHT milk: Difference between whole and skim milk', *J Dairy Res*, 60, 339–347.

LU D D and NIELSEN S S (1993), 'Assays for native plasminogen activators in bovine milk', *J Dairy Sci*, 76, 3362–3368.

LYSTER R L J (1964), 'The free and masked sulfhydryl groups of heated milk and milk powder and a new method for their determination', *J Dairy Res*, 31, 41–51.

MABBIT L A (1981), 'The significant metabolic activity of bacteria in raw milk', *Kiel Milchwirtsch Forschungber*, 33, 273–280.

MAGNUSSON M, CHRISTIANSSON A and SVENSSON B (2007), '*Bacillus cereus* spores during

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

housing of dairy cows: factors affecting contamination of raw milk', *J Dairy Sci*, 90, 2745–2754.

MAN D (2002), *Food Industry Briefing Series: Shelf Life*, Oxford, Blackwell Science.

MANJI B and KAKUDA Y (1988), 'The role of protein denaturation, extent of proteolysis and storage temperature on the mechanism of age gelation in a model system', *J Dairy Sci*, 71, 1455–1463.

MANJI B, KAKUDA Y and ARNOTT D R (1986), 'Effect of storage temperature on age gelation of ultra-high temperature milk processed by direct and indirect heating systems', *J Dairy Sci*, 69, 2994–3001.

MARCHAND S, HEYLEN K, MESSENS W, COUDIJZER K, DE VOS P, DEWETTINCK K, HERMAN L, DE BLOCK J and HEYNDRICKX M (2009a), 'Seasonal influence on heat-resistant proteolytic capacity of *Pseudomonas lundensis* and *Pseudomonas fragi*, predominant milk spoilers isolated from Belgian raw milk samples', *Environ Microbiol*, 11, 467–482.

MARCHAND S, VANDRIESCHE G, COOREVITS A, COUDIJZER K, DE JONGHE V, DEWETTINCK K, DE VOS P, DEVREESE B, HEYNDRICKX M and DE BLOCK J (2009b), 'Heterogeneity of heat-resistant proteases from milk *Pseudomonas* species', *Int J Food Microbiol*, 133, 68–77.

MARCO M L and WELLS-BENNIK M H J (2008), 'Impact of bacterial genomics on determining quality and safety in the dairy production chain', *Int Dairy J*, 18, 486–495.

MAUBOIS J L (1997), 'Current uses and future perspectives of MF technology in the dairy industry', *Bull Int Dairy Fed*, 320, 37–40.

MAYR R, GUTSER K, BUSSE M and SEILER H (2004), 'Gram positive non-sporeforming recontaminants are frequent spoilage organisms of German retail ESL (Extended Shelf Life) milk', *Milchwissenschaft-Milk Sci Inter*, 59, 262–266.

MCCARTHY C N, WOODS R G and BEACHAM I R (2004), 'Regulation of the *aprX-lipA* operon of *Pseudomonas fluorescens* B52: differential regulation of the proximal and distal genes, encoding protease and lipase, by *ompR-envZ*', *FEMS Microbiol Lett*, 241, 243–248.

MCKELLAR R C (1982), 'Factors influencing the production of extracellular proteinase by *Pseudomonas fluorescens*', *J Appl Bacteriol*, 53, 305–316.

MCKELLAR R C (1989), *Enzymes of Psychrotrophs in Raw Food*, Boca Raton, FL, CRC Press.

MCKELLAR R C and CHOLETTE H (1987), 'Effect on temperature shifts on extracellular proteinase-specific mRNA pools in *Pseudomonas* B52', *Appl Environ Microbiol*, 53, 1973–1976.

MEER R R, BAKKER J, BODYFELT F W and GRIFFITHS M W (1991), 'Psychrotrophic *Bacillus* spp. in fluid milk products: a review', *J Food Prot*, 54, 969–979.

MEGLIA G E, JENSEN S K, LAURIDSEN C and WALLER K P (2006), '$\alpha$-Tocopherol concentration and stereoisomer composition in plasma and milk from dairy cows fed natural or synthetic vitamin E around calving', *J Dairy Res*, 73, 227–234.

MEHTA R S (1980), 'Milk processed at ultra-high-temperatures – a review', *J Food Prot*, 43, 212–225.

MOLLER A B, ANDREWS A T and CHEESEMAN G C (1977), 'Chemical changes in ultra-heat-treated milk during storage. 2. Lactuloselysine and fructoselysine formation by Maillard reaction', *J Dairy Res*, 44, 267–275.

MONTANARI G, BORSARI A, CHIAVARI C, FERRI G, ZAMBONELLI C and GRAZIA L (2004), 'Morphological and phenotypical characterization of *Bacillus sporothermodurans*', *J Appl Microbiol*, 97, 802–809.

MORGAN D R, ANDERSON D L and HANKINSON C L (1971), 'Process for inhibiting staling of

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

milk prior to sterilization', US patent 3,615,617.

MORSE P M, JACKSON H, MCNAUGHTON C H, LEGGAT A G, LANDERKIN G B and JOHNS C K (1968), 'Investigation of factors contributing to the bacteria count of bulk tank milk. II. bacteria in milk from individual cows', *J Dairy Sci*, 51, 1188–1191.

MOSSEL D A A, CORRY J E L, STRUIJK C B and BAIRD R M (1995), *Essentials of the Microbiology of Foods: a Textbook for Advanced Studies*, Chichester, John Wiley & Sons.

MOYSSIADI T, BADEKA A, KONDYLI E, VAKIRTZI T, SAVVAIDIS I and KONTAMINAS M (2004), 'Effect of light transmittance and oxygen permeability of various packaging materials on keeping quality of low fat pasteurized milk: chemical and sensorial aspects', *Int Dairy J*, 14, 429–436.

NICODÈME J-P, GRILL G and GAILLARD J-L (2005), 'Extracellular protease activity of different *Pseudomonas* strains: dependence of proteolytic activity on culture conditions', *J Appl Microbiol*, 99, 641–648.

NICOLAOU N and GOODACRE R (2008), 'Rapid and quantitative detection of the microbial spoilage in milk using Fourier transform infrared spectroscopy and chemometrics', *Analyst*, 133, 1424–1431.

NOORDHUIZEN J, CANNAS DA SILVA J, BOERSEMA S-J and VIEIRA A (2008), *Applying HACCP-based Quality Risk Management on Dairy Farms*, Wageningen, the Netherlands, Wageningen Academic Publishers.

NOZIÈRE P, GRAULET B, LUCAS A, MARTIN B, GROLIER P and DOREAU M (2006), 'Carotenoids for ruminants: from forages to dairy products', *Animal Feed Sci Technol*, 131, 418–450.

PAPACHRISTOU C, BADEKA A, CHOULIARA I, KONDYLI E, ATHANASOULAS A and KONTOMINAS M (2006a), 'Evaluation of polyethylene terephthalate as a packaging material for premium quality whole pasteurized milk in Greece. Part I. Storage in the dark', *Eur Food Res Technol*, 223, 711–718.

PAPACHRISTOU C, BADEKA A, CHOULIARA I, KONDYLI E, KOURTIS L and KONTOMINAS M (2006b), 'Evaluation of polyethylene terephthalate as a packaging material for premium quality whole pasteurized milk in Greece. Part II. Storage under fluorescent light', *Eur Food Res Technol*, 224, 237–247.

PENG J S, TSAI W C and CHOU C C (2001), 'Surface characteristics of *Bacillus cereus* and its adhesion to stainless steel', *Int J Food Microbiol*, 65, 105–111.

PERKINS M L, ELLIOTT A J, D'ARCY B R and DEETH H C (2005), 'Stale flavour volatiles in Australian commercial UHT milk during storage', *Aust J Dairy Technol*, 60, 231–237.

PETA M E, LINDSAY D, BROZEL V S and VON HOLY A (2003), 'Susceptibility of food spoilage *Bacillus* species to chlorine dioxide and other sanitizers', *South African J Sci*, 99, 375–380.

PETTERSSON B, LEMBKE F, HAMMER P, STACKEBRANDT E and PRIEST F G (1996), '*Bacillus sporothermodurans*, a new species producing highly heat-resistant endospores', *Int J Syst Bacteriol*, 46, 759–764.

REIJ M W and DEN AANTREKKER E D (2004), 'Recontamination as a source of pathogens in processed foods', *Int J Food Microbiol*, 91, 1–11.

ROGINSKI H (2003), *Encyclopedia of Dairy Sciences*, London, Academic Press.

ROWE M T and GILMOUR A (1982), 'Growth, enzyme production and change in oxygen tension occurring during batch cultivation of psychrotrophic *Pseudomonas fluorescens* strains', *Milchwissenschaft*, 37, 597–600.

RYSER E T (2003), 'Pasteurization of liquid milk products', in Roginski J, Fuquay W and

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-eBooks.com

Fox P F, *Encyclopedia of Dairy Sciences*, London, Academic Press, 2232–2237.

RYSSTAD G and KOLSTAD J (2006), 'Extended shelf-life milk – advances in technology', *Int J Dairy Techn*, 59, 85–96.

RYSSTAD G, EBBESEY A and EGGESTAD J (1998), 'Sensory and chemical quality of UHT milk stored in paperboard cartons with different oxygen and light barriers', *Food Additives Contaminants*, 15, 112–122.

RYU J H and BEUCHAT L R (2005), 'Biofilm formation and sporulation by *Bacillus cereus* on a stainless steel surface and subsequent resistance of vegetative cells and spores to chlorine, chlorine dioxide, and a peroxyacetic acid-based sanitizer', *J Food Prot*, 68, 2614–2622.

SAGRIPANTI J L and BONIFACINO A (1999), 'Bacterial spores survive treatment with commercial sterilants and disinfectants', *Appl Environ Microbiol*, 65, 4255–4260.

SANDROU D K and ARVANITOYANNIS S (2000), 'Implementation of hazard analysis critical control points (HACCP) to the dairy industry: current status and perspectives', *Food Rev Inter*, 16, 77–111.

SATTAR A, DEMAN J M and ALEXANDER J C (1977), 'Light-induced degradation of vitamins. I. Kinetic studies on riboflavin decomposition in solution', *Canadian Institute Food Sci Technol J*, 10, 61–64.

SCHELDEMAN P, HERMAN L, GORIS J, DE VOS P and HEYNDRICKX M (2002), 'Polymerase chain reaction identification of *Bacillus sporothermodurans* from dairy sources', *J Appl Microbiol*, 92, 983–991.

SCHELDEMAN P, HERMAN L, FOSTER S and HEYNDRICKX M (2006), '*Bacillus sporothermodurans* and other highly heat-resistant spore formers in milk', *J Appl Microbiol*, 101, 542–555.

SCHINGOETHE D J, PARSONS J G, LUDENS F C, TUCKER W L and SHAVE H J (1978), 'Vitamin E status of dairy cows fed stored feeds continuously or pastured during summer', *J Dairy Sci*, 61, 1582–1589.

SCHRÖDER M J A (1983), 'Light and copper catalysed oxidized flavours in stored milk', *J Soc Dairy Technol*, 36, 8–12.

SCHRÖDER M J A, SCOTT K J, BLAND K J and BISHOP D R (1985), 'Flavour and vitamin stability in pasteurized milk in polyethylene-coated cartons and in polyethylene bottles', *J Soc Dairy Technol*, 38, 48–52.

SILLANKORVA S, NEUBAUER P and AZEREDO J (2008), '*Pseudomonas fluorescens* biofilms subjected to phage phiIBB-PF7A', *BMC Biotechnol*, 8, 79.

SIMÕES M, SIMÕES L C and VIEIRA M J (2009), 'Species association increases biofilm resistance to chemical and mechanical treatments', *Water Res*, 43, 229–237.

SIMON M and HANSEN A P (2009), 'Effect of various dairy packaging materials on the shelf-life and flavor of ultrapasteurized milk', *J Dairy Sci*, 84, 784–791.

SIMOPOULOS A P (2002), 'The importance of the ratio of omega-6/omega-3 essential fatty acids', *Biomed Pharmacother*, 56, 365–379.

SINGH R P and HELDMAN D R (2009), *Introduction to Food Engineering*, Amsterdam, Elsevier.

SKIBSTED L H (2000), 'Light-induced changes in dairy products', *Bull Int Dairy Fed*, 346, 4–9.

SLAGHUIS B A, TE GIFFEL M C, BEUMER R R and ANDRÉ G (1997), 'Effect of pasturing on the incidence of *Bacillus cereus* spores in raw milk', *Int Dairy J*, 7, 201–205.

SLOTS T, SKIBSTED L H and NIELSEN J H (2007), 'The difference in transfer of all-rac-$\alpha$-tocopherol stereo-isomers to milk from cows and the effect on its oxidative stability', *Int Dairy J*, 17, 737–745.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-eBooks.com

SLOTS T, SORENSEN J and NIELSEN J H (2008), 'Tocopherol, carotenoids and fatty acid composition in organic and conventional milk', *Milchwissenschaft*, 63, 352–355.

SLOTS T, BUTLER G, LEIFERT C, KRISTENSEN T, SKIBSTED L H and NIELSEN J H (2009), 'Potentials to differentiate milk composition by different feeding strategies', *J Dairy Sci*, 92, 2057–2066.

SORHAUG T and STEPANIAK L (1997), 'Psychrotrophs and their enzymes in milk and dairy products: quality aspects', *Trends Food Sci Technol*, 8, 35–41.

ST-LAURENT A-M, HIDIRIGLOU M, SNODDON M and NICHOLSON J W G (1990), 'Effect of $\alpha$-tocopherol supplementation to dairy cows on milk and plasma $\alpha$-tocopherol concentrations and on spontaneous oxidized flavor in milk', *Can J Animal Sci*, 70, 561–570.

SUN H, KAWAMURA S, HIMOTO J, ITOH K, WADA K and KIMURA T (2008), 'Effects of ohmic heating on microbial counts and denaturation of proteins in milk', *Food Sci Technol Res*, 14, 117–123.

SUTHERLAND J P, VARNAM A H and EVANS M G (1986), *A Colour Atlas of Food Quality Control*, London, Wolfe Publishing.

TAUVERON G, SLOMIANNY C, HENRY C and FAILLE C (2006), 'Variability among *Bacillus cereus* strains in spore surface properties and influence on their ability to contaminate food surface equipment', *Int J Food Microbiol*, 110, 254–262.

TE GIFFEL M C, BEUMER R R, BONESTROO M H and ROMBOUTS F M (1996a), 'Incidence and characterization of *Bacillus cereus* in two dairy processing plants', *Neth Milk Dairy J*, 50, 479–492.

TE GIFFEL M C, BEUMER R R, LEIJENDEKKERS S and ROMBOUTS F M (1996b), 'Incidence of *Bacillus cereus* and *Bacillus subtilis* in foods in the Netherlands', *Food Microbiol*, 13, 53–58.

TERNSTRÖM A, LINDBERG A M and MOLIN G (1993), 'Classification of the spoilage flora of raw and pasteurized bovine milk, with special reference to *Pseudomonas* and *Bacillus*', *J Appl Bacteriol*, 75, 25–34.

THOMAS E L, BURTON H, FORD J E and PERKIN A G (1975), 'Effect of oxygen-content on flavor and chemical changes during aseptic storage of whole milk after ultra-high-temperature processing', *J Dairy Res*, 42, 285–295.

THOMAS S B (1966), 'Sources, incidence and significance of psychrotrophic bacteria in milk', *Milchwissenschaft*, 21, 270–275.

THOMAS S R, NEUZIL J and STOCKER R (1996), 'Cosupplementation with coenzyme Q prevents the prooxidant effect of $\alpha$-tocopherol and increases the resistance of LDL to transition metal-dependent oxidation initiation', *Arteriosclerosis Thrombosis Vascular Biology*, 16, 687–696.

VAEREWIJCK M J, DE VOS P, LEBBE L, SCHELDEMAN P, HOSTE B and HEYNDRICKX M (2001), 'Occurrence of *Bacillus sporothermodurans* and other aerobic spore-forming species in feed concentrate for dairy cattle', *J Appl Microbiol*, 91, 1074–1084.

VAN ASSELT A J, SWEERE A P J, ROLLEMA H S and DE JONG P (2008), 'Extreme high-temperature treatment of milk with respect to plasmin inactivation', *Int Dairy J*, 18, 531–538.

VAN DEN BROECK D., BLOEMBERG V G and LUGTENBERG B (2005), 'The role of phenotypic variation in rhizosphere *Pseudomonas* bacteria', *Environ Microbiol*, 7, 1686–1697.

VANLOOSDRECHT M C M, LYKLEMA J, NORDE W and ZEHNDER A J B (1989), 'Bacterial adhesion – a physicochemical approach', *Microb Ecol*, 17, 1–15.

VISSER S (1981), 'Proteolytic enzymes and their action on milk proteins. A review', *Neth Milk Dairy J*, 35, 65–88.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

VISSERS M M M, TE GIFFEL M C, DRIEHUIS F, DE JONG P and LANKVELD J M G (2007a), 'Minimizing the level of *Bacillus cereus* spores in farm tank milk', *J Dairy Sci*, 90, 3286–3293.

VISSERS M M M, TE GIFFEL M C, DRIEHUIS F, DE JONG P and LANKVELD J M G (2007b), 'Predictive modeling of *Bacillus cereus* spores in farm tank milk during grazing and housing periods', *J Dairy Sci*, 90, 281–292.

WADSWORTH K D and BASETTE R (1985), 'Effect of oxygen on development of off-flavours in ultra-high-temperature milk', *J Food Prot*, 48, 487–493.

WASHAM C J, OLSON H C and VEDAMUTHU E R (1977), 'Heat-resistant psychrotrophic bacteria isolated from pasteurized milk', *J Food Prot*, 40, 101–108.

WEISS W P and WYATT D J (2003), 'Effect of dietary fat and vitamin E on $\alpha$-tocopherol in milk from dairy cows', *J Dairy Sci*, 86, 3582–3591.

WERNER B G and HOTCHKISS J H (2006), 'Continuous flow nonthermal $CO_2$ processing: the lethal effects of subcritical and supercritical $CO_2$ on total microbial populations and bacterial spores in raw milk', *J Dairy Sci*, 89, 872–881.

WIJMAN J G E, DE LEEUW P P L A, MOEZELAAR R, ZWIETERING M H and ABEE T (2007), 'Air–liquid interface biofilms of *Bacillus cereus*: Formation, sporulation, and dispersion', *Appl Environ Microbiol*, 73, 1481–1488.

WOLFSON L M and SUMNER S S (1993), 'Antibacterial activity of the lactoperoxidase system: a review', *J Food Prot*, 56, 887–892.

WOODS R G, BURGER M, BEVEN C A and BEACHAM I R (2001), 'The *aprX-lipA* operon of *Pseudomonas fluorescens* B52: a molecular analysis of metalloprotease and lipase production', *Microbiol*, 147, 345–354.

WU X Y, WALKER M, VANSELOW B, CHAO R L and CHIN J (2007), 'Characterization of mesophilic bacilli in faeces of feedlot cattle', *J Appl Microbiol*, 102, 872–879.

ZHANG Y Q C, RONIMUS R S, TURNER N, ZHANG Y and MORGAN H W (2002), 'Enumeration of thermophilic *Bacillus* species in composts and identification with a random amplification polymorphic DNA (RAPD) protocol', *Syst Appl Microbiol*, 25, 618–626.

ZHOU G P, LIU H Z, HE J, YUAN Y M and YUAN Z M (2008), 'The occurrence of *Bacillus cereus*, *B. thuringiensis* and *B. mycoides* in Chinese pasteurized full fat milk', *Int J Food Microbiol*, 121, 195–200.

ZYGOURA P, MOYSSIADI T, BADEKA A, KONDYLI E, SAVVAIDIS I and KONTAMINAS M (2004), 'Shelf-life of whole pasteurized milk in Greece: effect of packaging material', *Food Chem*, 87, 1–9.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

# 5

# Effects of packaging on milk quality and safety

**M. Kontominas, University of Ioannina, Greece**

**Abstract:** Packaging is a major factor contributing to the quality and safety of milk. This chapter reviews contemporary milk packaging materials including metals, glass, plastics and composites. Factors related to packaging affecting milk shelf-life and safety including microorganisms, temperature, light, oxygen, permeability and migration are also discussed. Finally, environmental issues regarding the management of packaging materials are addressed.

**Key words:** packaging, milk, quality, safety.

IP Address: 129.132.208.100

## 5.1 Introduction

Food packaging is an integral part of food processing operations and food preservation. It serves a number of different functions including preservation, containment, convenience and communication; among these, preservation is by far its most important function. Packaging protects the contents against environmental, physical, chemical and mechanical hazards (light, oxygen, moisture, etc.), loss of desirable flavor compounds or pickup of undesirable odors, as well as contamination from microorganisms, insects or rodents during storage and distribution. In addition to the above, an effective packaging material should fulfill many other requirements including recyclability or reuse, compatibility with the food it contains, low cost, tamper resistance, esthetics, non-toxicity, machinability and functionality in terms of shape, size and disposability (Paine 1996). All these requirements, which hold for all

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

foodstuffs, are particularly important for milk, a complex mixture of water, lipids, proteins, carbohydrates and minerals. Milk, due to its composition, is a highly perishable product of great spoilage potential resulting in rapid deterioration of quality and safety. Quality deterioration may be related to (1) the effect of oxygen and light, causing autooxidation and light-induced oxidation, respectively, and (2) psychrotrophic bacterial activity resulting in undesirable flavor changes in the product. Product safety may be affected either by incomplete destruction of pathogens transferred to milk through the animal or by cross-contamination at any stage after heat treatment with a particular pathogen. Packaging offers effective protection from such hazards (Skibsted 2000, Vassila *et al.* 2002).

Milk is processed into a variety of products including pasteurized, ultra-pasteurized, UHT, microfiltered, condensed, cultured, flavored milk and milk powder, all having different expected shelf-life and thus different packaging requirements. Development of packaging materials for milk products, through time, focused on parameters such as the nature of the product (i.e. liquid versus powder), desired shelf-life, storage conditions and cost. Selection of a particular packaging material for a particular milk product requires in-depth knowledge of product properties, deterioration mechanisms, transportation hazards, market and distribution requirements, as well as specific properties of available packaging materials and machinery.

Contemporary milk packaging materials include metals, glass, plastics, paperboard, fiberboard and composites. However, before discussing specific milk packaging applications it is worth mentioning that there are three levels of packaging: primary, secondary and tertiary. A primary packaging material is in direct contact with the milk product. A secondary package usually contains several primary packages and provides the mechanical strength for stacking primary packages in the warehouse. A tertiary package usually contains several secondary packages and holds these together during distribution. With respect to quantity of milk contained within the package, packaging may be categorized as retail or bulk packaging (Grønborg 1995).

IP Address: 129.132.208.100

## 5.2 Types of packaging materials and their applications

### 5.2.1 Primary packaging materials

*Tin cans*

Tin cans have been widely used in the past and are still used today for the production of evaporated, sterilized milk in capacities of 395–410 g and 170 g. Such retail cans are usually equipped with an easy-open top. Raw milk is first evaporated at approximately 60–65ºC under reduced pressure, then homogenized to prevent it from separating under storage and subsequently cooled. The evaporated milk is poured into cans sealed and sterilized at 121ºC for about 10–15 min. Finally the product is cooled and labeled. As a result of thermal processing, evaporated milk possesses a characteristic ‘caramel’ color and ‘cooked’ flavor.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

Condensed milk (evaporated milk with the addition of sugar) is also packaged in similar-sized tin cans.

The use of tin cans has many advantages. They provide a truly hermetic seal, an excellent protection from gases, light, moisture and microorganisms, they stack easily, they are tamper proof and relatively inexpensive. Disadvantages include generally low quality of final product, due to severe heat treatment, difficulty in opening, relatively large weight of container, inability for microwave processing, etc.

The most common type of metal container is the three-piece can, consisting of two ends and one cylindrical body (usual can dimensions: diameter 75 mm, height 105 mm; or diameter 65 mm, height 64 mm). The cans are made of tin plate, which is composed of a thick layer of steel with tin added on both sides. The tin layers protect the steel from corrosion. Additional protection to the product is provided by application of synthetic polymeric resins known as lacquers to the internal can walls, but also externally, consisting usually of epoxy-phenolic or epoxy-amino polyester resin (Brody and Marsh 1996).

*Aluminum cans*

Aluminum cans are two-piece metal containers, one piece making up the can body and the bottom end and the second piece making up the top end. Aluminum cans are also coated for protection against corrosion. They are used for packaging of vitamin-fortified milk for youngsters, and flavored milk (e.g. coffee, cinnamon, caramel, nut or vanilla flavored milk) in 330 ml containers. Flexible aluminum containers are also used in single-serving coffee creamers. In such applications the container body is made of aluminum coated with polyethylene while the top web is usually a multilayer material composed of either an all-plastic or a plastic plus aluminum laminate. Advantages of aluminum cans over tin cans include lighter weight, better esthetic appeal and easier recyclability. They do, however, have a higher cost than tin cans of comparable size (Robertson 1993).

IP Address: 129.132.208.100

*Glass bottles*

Glass milk bottles are classified into two groups: bottles with narrow necks (neck diameter 26 mm) and those with wide necks (neck diameter 35–40 mm). Glass is the most inert of all packaging materials and provides ultimate protection from oxygen, moisture and microorganisms. When colored properly (blue, amber, green and to a lesser degree white) it can partly protect milk from harmful UV light. Sealing of glass bottles for milk packaging is usually achieved with aluminum foil caps. Most glass bottles are returnable, making on average 30 trips (FAO 2007).

Major disadvantages of glass are fragility and heavy weight. A fraction of pasteurized, ultrapasteurized and sterilized milk is sold in glass bottles today in several countries including Great Britain, Sweden, Greece, Cyprus, etc. Glass bottles are usually placed in plastic crates (secondary packaging) bearing internal divisions so that bottles are not in contact with one another, so as to

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

minimize risk of breakage. In turn, crates filled with bottles are stacked five or six high and palletized on, for example, standard europallets (800 × 1200 mm) made of wood or plastic. Palletized crates are then wrapped with plastic (polyethylene) film (tertiary packaging) so as to remain safe during movement by forklift trucks or during transportation by truck, ship or train. Typical 1-liter bottle dimensions for pasteurized milk are 89 mm (base diameter), 35–40 mm (neck diameter) and 267 mm (height); and for sterilized milk 89 mm (base diameter), 26 mm (neck diameter) and 294 mm (height). Bottles used for in-bottle sterilization of milk have narrow necks to ensure a more effective seal as compared to pasteurized milk. Prefabricated crown seals are used to seal these bottles. Such bottles have to withstand the heat sterilization process and subsequent cooling. During sterilization the milk expands more than the bottle and the air in the bottle headspace is compressed, resulting in pressure build-up inside the bottle which exceeds the external pressure. Upon subsequent cooling milk contracts, creating a partial vacuum in the bottle headspace. Such a vacuum may cause contamination through the seal between bottle and cap. It is for this reason that seals must be fully airtight. As plastics technology advances, partial replacement of glass containers by plastics has occurred in various liquid food packaging applications including milk (INEOS Polyolefins 2007).

*All-plastic containers*

Plastics are composed of long-chain polymers to which a number of commercial additives are added to improve physical, chemical and mechanical properties of the end product. General advantages of plastics as packaging materials are light weight, non-fragility, good moisture and gas barrier properties, good sealant properties, recyclability, puncture resistance, etc. Among the disadvantages of plastics are migration, that is the transfer of low molecular weight compounds from the container to the product, and flavor scalping, that is the absorption of product flavor compounds by the container. The main plastics used in milk packaging are high density polyethylene (HDPE), polyethylene terephthalate (PET), polycarbonate (PC), high impact polystyrene (HIPS) and low density polyethylene (LDPE).

IP Address: 129.132.208.100

HDPE jugs

Jugs of various capacities between ¼ and 1 gallon are widely used containers for milk packaging in several countries including the USA, Canada, Great Britain, etc. Unpigmented HDPE bottles transmit 58–79% of the incident light in the wavelength range 350–800 nm. Light transmission can be reduced by pigmenting HDPE with titanium dioxide (1–2%), producing an opaque bottle at wavelengths below 390 nm. HDPE jugs are blow-molded to provide a thin-walled, light and tough container. An advantage of this type of packaging, especially in the ½ and 1 gallon size, is the handle on the bottle which makes it more convenient to hold than the respective paperboard carton. Modern dairies blow-mold their own HDPE jugs to avoid shipping costs and storage space in the dairy plant. HDPE jugs are used for pasteurized full-fat, semi-skimmed and

© Woodhead Publishing Limited, 2010

Downloaded From Vet-eBooks.com

skimmed milk. Recently multilayer HDPE bottles have been introduced into the market for ultrapasteurized and UHT milk, using coextrusion technology. In the case of ultrapasteurized milk a three-layer HDPE bottle is used consisting of an inside and an outside white layer (2% $TiO_2$) and a middle black layer (2% carbon black). In the case of sterilized and UHT milk, either the barrier of the three-layer container is enhanced by the addition of a PVC–PVdC copolymer coating (5–6 $\mu$m) or a five-layer HDPE container is used consisting of an outside HDPE white layer (2% $TiO_2$), an adhesive layer, a middle black EVOH (2% carbon black) layer, an adhesive layer and an inside white HDPE layer (2% $TiO_2$) (Mottar 1987, Karatapanis *et al.* 2006, INEOS Polyolefins 2007).

PET bottles

PET bottles are blow-molded from PET preforms in sizes ranging from ½ liter to 2 liters. They are superior to HDPE bottles in terms of their mechanical and optical properties, their lower flavor-scalping potential and their substantially lower gas permeability values, i.e. the oxygen transmission rate at 4ºC and 50% RH of a commercial one-pint PET bottle is 19 $\mu$L/day compared to 390–460 $\mu$L/day for a commercial one-pint HDPE bottle (Van Aardt *et al.* 2001).

Due to the almost complete transparency of PET to light, milk bottles are either labeled or, even better, sleeved using thermoshrinkable polypropylene (PP) labels. Today most PET bottles are wide-necked (diameter 35–40 mm) and sealed with rigid PP screw caps. Besides full-fat, semi-skimmed and skimmed milk, PET bottles are used to package flavored milks (vanilla, chocolate, strawberry, etc.) (Dimmick 2007), cultured milk, ultrapasteurized and microfiltered milk. Although PET bottles used by the dairy industry are single-use, other industries such as the carbonated soft drinks industry are considering multi-use PET bottles (Demertzis *et al.* 1997).

IP Address: 129.132.208.100

Polycarbonate bottles

Polycarbonates (PC) are polyesters of unstable carbonic acid. They are formed through the reaction of bisphenol A and phosphene. PC has high temperature resistance, high impact strength and clarity and is currently being used for the production of multi-use baby milk bottles, which are sterilized before each use. In a study (Landsberg *et al.* 1977) where glass, polyethylene and polycarbonate multi-use milk containers were treated with 19 common household chemicals to simulate consumer abuse, glass was found to be the most resistant to retention of contaminants used.

High impact polystyrene (HIPS)

A small but important market exists for the one-ounce portions of cream served with coffee in restaurants (coffee creamers). These are made of a thermoformed HIPS cup lidded with aluminum foil coated with either LDPE or ethylene vinyl acetate (EVA) to achieve thermal sealing. More recently, aseptically processed and packaged coffee creamers have been introduced into the market, extending

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

product shelf-life to about 100 days. A factor limiting the shelf-life of such products is the poor oxygen barrier of HIPS.

LLDPE/LDPE pouches (sachets)

This concept was first developed in Canada in the late 1960s. Pillow-shaped pouches for milk are produced by feeding a linear low density polyethylene film (75–80 $\mu$m) to a form–fill–seal machine creating a 'tube'. The tube bottom is heat-sealed, the milk is pumped in and the top sealed. The process is continuous without interrupting the flow of milk. Milk is dispensed from the pouch by placing it in a pitcher and clipping off the top pouch corner with scissors. A disadvantage of the pouch is that it cannot be reclosed, exposing the milk to odor absorption in the refrigerator. LLDPE is the resin preferred for such an application, possessing high melt strength, excellent seal integrity and toughness to withstand tears and pinholes. The pouch material should be colored to reduce light transmission. For home use a combination of two pouches is used: an outer made of either LDPE or LLDPE and an inner made of LLDPE. The double-ply structure is used to prevent leakage, which is unlikely to be present in both layers. Alternatively LLDPE may be coextruded with an ultra low density polyethylene (ULDPE) for improved sealant performance (Falla 2004). Sizes available range from ½ liter to 2 liters. Dimensions for a one-liter sachet are 220–240 nm (length) by 120–140 mm (width). For institutional use, such as in restaurants and cafeterias, pouch capacity may rise to 20 liters. In cases of such large milk volumes the PE pouch is supported by an injection-molded HDPE crate. Pouches have been used for the packaging of pasteurized milk in countries such as India, Mexico, etc.

In a study on light-induced quality deterioration of milk (Sattar and deMan 1973) of the four different packaging materials used – clear PE pouch, coextruded polyethylene pouch (outer white, middle black, inner white PE layer), paperboard carton and returnable HDPE jug – off-flavor was detected in all containers except in the coextruded multilayer pouch. In another study on pasteurized milk (Vassila *et al.* 2002), it was shown that a coextruded pouch material (LDPE + 2% $TiO_2$/LDPE + 2% $TiO_2$/LDPE + 4% carbon black/LDPE + 2% $TiO_2$/LDPE + 2% $TiO_2$), 110 $\mu$m thick, showed equal retention of vitamins A and $B_2$ to the paperboard carton after 7 days at 4ºC.

*Paperboard based containers*

These are multilayer containers either rectangular with a gable top, rectangular with a flat top or tetrahedral in shape. The material used for pasteurized milk is polyethylene extrusion-coated paperboard in the form of PE/paperboard/PE. The thickness of the paperboard is usually 420–490 $\mu$m while the thickness of the two PE layers is 45–55 $\mu$m. Polyethylene is used externally to provide protection from moisture and indirectly for mechanical integrity, and internally to avoid interaction of milk with the paperboard and to provide effective heat sealing. For UHT milk packaging applications, aluminum foil is added as an extra layer between the paperboard and the internal PE layer (PE/paperboard/PE/aluminum

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

foil/PE/PE) to provide the required barrier in the container. The innermost PE layer is applied at a lower temperature than is the next layer outwards. This minimizes the tendency for PE degradation products formed at high temperatures to diffuse into the milk and alter its taste. Rectangular gable-top blank containers 0.25–2 liters in capacity are pre-cut and pre-creased ready to be formed into milk containers based on the erect form–fill–seal principle. The blank type container is manufactured by first forming a cylindrical tube from a reel of material, seaming it longitudinally, filling it with the milk and then making transverse seals. The whole operation uses patented technology. Gable-top containers are used for pasteurized milk (whole, semi-skimmed and skimmed) while brick and tetrahedron type containers are used for UHT milk.

Farrer (1983) compared UHT milk packaged in polyethylene-coated paperboard cartons with and without an aluminum foil layer. Results showed that $O_2$ in the milk packaged in the container with aluminum foil remained almost unchanged at 1 ppm after 44 days while in the milk packaged in the container without aluminum foil, $O_2$ rose to 8–9 ppm after only a few days. Milk in the first case was sensorily acceptable for two months even at 38ºC, while in the latter case milk was acceptable for only three weeks at 15ºC.

Simon and Hansen (2001a) used (1) standard milk board, (2) standard board including an ethylene vinyl alcohol (EVOH) barrier layer, and (3) standard board including an aluminum foil layer to package ultrapasteurized milk. Quality was assessed over a period of 15 weeks. It was found that the flavor of milk packaged in standard board deteriorated at a faster rate than milk packaged in barrier and foil boards. At week 6 of storage, a slightly 'cardboardy' flavor was detected in milk packaged in standard board and a slightly 'cooked' flavor was detected in milk packaged in barrier and foil boards. The cardboard flavor intensified with storage time while all the cooked flavor dissipated at week 10 of storage.

IP Address: 129.132.208.100

*Fiberboard cans/composites*

Such containers are usually three-piece cans made of fiberboard based materials making up the composite can body while top and bottom pieces are usually made of aluminum. The can body comprises Kraft plies which are wound either spirally or convolutely around a metal mandrel. Companies such as Dole have used such containers for aseptically packaged milk in the US.

### 5.2.2 Secondary and tertiary packaging materials

The following materials may be used for the construction of either secondary or tertiary packages.

*Fiberboard–corrugated board*

In principle this is a composite material in which fluting is glued to flat sheets of paper (linerboard). Linerboard is made of either Kraft or Test. Kraft liners are made of virgin fibers whereas Test liners are made mostly of recycled fibers

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

from waste board and paper. An extensive range of corrugated board types is available. In its simplest form, corrugated board is constructed from one layer of fluting medium and one layer of linerboard. This type of corrugated board is used for cushioning. The range of corrugated board extends from double-faced board composed of one layer of fluting medium and two layers of linerboard to triple-wall board composed of three fluting layers sandwiched between four layers of linerboard. There are five main flute constructions available, namely A, C, B, E and F. Each flute construction is related to the number of individual flutes per meter of corrugated board: A (110–125 flutes/m), C (120–145 flutes/m), B (150–185 flutes/m), E (320 flutes/m) and F (420 flutes/m).

If the packaging system needs to be crush resistant, E flute is the preferred material and A flute is the worst. If cushioning is desired the reverse is true. Linerboard ranges in weight between 125 and 450 g/m$^2$ and fluting medium between 110 and 160 g/m$^2$. Corrugated board in the form of rectangular boxes (dimensions 50 cm length × 30 cm width × 22 cm height) is being used in the dairy industry to pack cylindrical cans of condensed milk, or bags, usually of capacity between 3 and 20 liters in UHT milk bag-in-box packaging applications.

*Paperboard composites*

Composites are occasionally used for secondary packaging applications. A typical example of such an application is the packaging of milk powders in a polyethylene bag contained for protection purposes within a composite container. As the name implies, composites are usually paperboard based but may involve other materials such as aluminum and/or plastics.

IP Address: 129.132.208.100

Plastics

Plastics used by the dairy industry as materials for secondary and tertiary packaging include polyethylene, polypropylene, polystyrene, polyvinyl chloride and certain copolymers. These are formed into films, sacks, crates and trays.

Films

Stretch or shrink films are made of low density polyethylene (LDPE). LDPE has a density of 0.915–0.925 g/ml and a crystallinity between 55 and 70%. For the production of shrink film LDPE resin is extruded and forced through an annular die (film blowing process). By 'drawing' the blown film off more rapidly than it passes through the extrusion die and cooling quickly, orientation is 'built in'. This orientation relaxes when the film passes through a hot air oven and the film shrinks, 'embracing' the product. Shrinkability is commercially coded as follows:

- Shrinkability in the machine direction:

$$\frac{w}{(30-40\%)}\ \frac{x}{(40-50\%)}\ \frac{y}{(50-60\%)}\ \frac{z}{(>60\%)}$$

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

- Shrinkability in the cross-direction:

$$\frac{SA}{(<10\%)}\ \frac{SB}{(10-20\%)}\ \frac{SC}{(20-35\%)}\ \frac{SD}{(>35\%)}$$

where $w$ = shrinkage category $w$, $x$ = shrinkage category $x$, $y$ = shrinkage category $y$, $z$ = shrinkage category $z$, $SA$ = shrinkage category $A$, $SB$ = shrinkage category $B$, $SC$ = shrinkage category $C$, $SD$ = shrinkage category $D$.

The dairy industry generally uses LDPE 30 and 60 $\mu$m in thickness. LDPE may be used also as stretch film based on a similar principle as shrink-wrapping, except that a lower temperature air tunnel is utilized. The film, usually between 35 and 45 $\mu$m thick, is stretched by 25–30% over the collated product (i.e. milk cartons or brick-type UHT containers), sealed and either left to return to its original length or passed through a short hot air tunnel assisting its relaxation process. Stretch and shrink films often used are made of PVC. PVC shrink films are generally thinner than the respective PE films. Commercial thickness ranges between 13 and 38 $\mu$m, but 50 $\mu$m thick PVC film may also be used. PVC has a higher clarity than PE and thus is selected for shrink-wrapping presentation packs. Shrinkability, in both machine and cross-directions, may be between 10% and 35%. Selection of PE or PVC for shrink-wrapping is made after considering its clarity at a given thickness. PVC has generally a higher yield at a lower thickness (13–18 $\mu$m vs. 25–30 $\mu$m for PE). PVC stretch film may be used to unitize pallets just as PE stretch film. Its major advantage over PE stretch film is its superior clarity, although in most stretch-wrap applications clarity is not important (Grønborg 1995). PVC film thickness for stretch-wrapping applications is between 10 and 40 $\mu$m.

IP Address: 129.132.208.100

Crates and drums

Crates may be made from timber or metal but nowadays plastics dominate in crate construction. They are manufactured by injection molding of either HDPE or PP. Selection between the two polyolefins depends on the end use of the crate, i.e. HDPE shows an excellent resistance at temperatures down to −30ºC, whereas PP is not recommended for temperatures below 0ºC. Under load, HDPE crates should not exceed 60ºC while PP crates should not exceed 100ºC. The range of crate sizes is extensive and their design is such that they will stack when full and nest when empty. Exagonal prism-shaped crates are used for the distribution of tetrahedral cartons for both pasteurized and UHT milk (i.e. ½ liter capacity) holding 18 cartons per crate. Rectangular crates are used for the distribution of pasteurized milk in bottles (glass or PE) or sachets of ½–1 liter capacity holding 16–20 bottles per crate.

Crates in both cases are stacked on pallets (standard europallets of dimensions 800 × 1200 mm made of timber or plastic-HDPE). There are no international standards for the dimensions of pasteurized milk bottles and crates. Drums are made of either plastic (HDPE) or fiberboard and vary in capacity up to 1200 liters. Such drums are used for the distribution of UHT milk packaged in bags.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

Sacks

Woven sacks made of PP are used as secondary packaging materials and their use is limited to milk powder containers (>500 kg). The open texture of sacks requires the use of an inner liner made usually of PE. Such sacks are used for the distribution of milk powders, providing an alternative to PE-lined paper sacks. The major feature of such woven PP sacks is their great strength.

Paper sacks

Paper sacks made of either chemically (bisulfite or sulfate process) or mechanically treated wood fibers are probably one of the oldest forms of packaging, having been used by the dairy industry for the packaging of milk and milk powders for many decades. Three- to five-ply sacks are generally used for milk powders. A typical lightweight sack is composed of three plies (80 g/m$^2$ Kraft; 23 g/m$^2$ PE; 80 g/m$^2$ Kraft). Sacks are sealed by stitching with a support paper such as crepe. For intervention storage, complexity in the structure of sacks increases while paper sacks are lined internally with PE 0.06–0.12 mm thick.

## 5.3 Factors related to packaging affecting milk shelf-life and safety

Milk is an excellent medium for growth of many microorganisms. It is high in moisture, has a close to neutral pH, and is rich in proteins, butterfat and milk sugar. All three classes of nutrients may be used by microorganisms and their enzymes to deteriorate quality and influence the safety of milk. In most cases the result is the development of various taints and off-flavors. Milk is therefore a rather complex and unstable food substrate, undergoing a number of spontaneous changes not only at room temperature but also under chill storage. In order to stabilize milk, the product is heat treated to destroy microorganisms (pasteurization, sterilization), packaged to avoid post-thermal treatment contamination (PTTC) and kept either under chilled storage (in the case of pasteurized or ultra-pasteurized milk) or at room temperature (in the case of sterilized milk). Thus microbial load and storage temperature play a key role in packaged milk quality and safety. In addition to the protection provided to milk against microorganisms, packaging must protect milk from atmospheric oxygen and moisture (in the case of milk powders) but also from light and pick-up of undesirable odors from the environment. Lastly, packaging should be substantially inert in terms of interaction with milk in order to avoid migration phenomena (transfer of packaging components into the milk) or flavor-scalping phenomena (absorption of desirable flavor components of milk by the packaging material). Given the significance of all the above factors on milk quality and safety, they will be dealt with below in more detail.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

### 5.3.1 Effect of microorganisms and temperature

The main purpose of heat treatment of milk is to render it safe for human consumption and to increase its shelf-life. The most common pathogenic organisms likely to occur in milk are destroyed by heat treatment. Heat treatment also inactivates enzymes that may deteriorate milk flavor. Among the most heat-resistant non-spore forming organisms found in milk are *Mycobacterium tuberculosis* and *Coxiella burnetii* (Cerf and Condron 2006) which are used as index microorganisms in order to achieve complete safety of milk. Thus, any heat treatment capable of destroying these organisms is assumed to destroy all other pathogens in milk. Heat treatment of milk is applied on the basis of time–temperature combinations. The choice of a particular time–temperature combination is a matter of optimizing destruction of microorganisms while at the same time retaining quality of the product. The most common thermal treatments applied to fluid milk are low temperature, long time (LTLT) pasteurization (63–65ºC, 30 min), high temperature, short time (HTST) pasteurization (72–75ºC, 10–15 s), ultra-high pasteurization (UP) (115–121ºC, 2–4 s), ultra high temperature (UHT) sterilization (135–150ºC, 2–4 s), and sterilization (115–121ºC, 10–15 min). The shelf-life of product is 5–10 days for pasteurized milk under refrigeration, 15–20 days for ultra-pasteurized milk under refrigeration, 2–6 months for UHT sterilized milk combined with aseptic packaging at room temperature, and over a year for sterilized milk at room temperature. Bactofuged and microfiltered pasteurized milk has a shelf-life exceeding 20 days under refrigeration (Papachristou *et al.* 2006a). Heat treatments in excess of pasteurization result in changes in milk properties including partial denaturation of proteins, lactose caramelization, development of 'caramelized' or 'cooked' flavor, darkening of color and destruction of heat-sensitive vitamins.

IP Address: 129.132.208.100

Gruetzmacher and Bradley (1999) investigated shelf-life extension of pasteurized milk and concluded that cartons for using mandrels, filling heads and airborne microorganisms were sources of contamination during the filling process. Eliminating sources of post-pasteurization contamination and proper cleaning followed by sanitizing with chlorine significantly increased milk shelf-life in paperboard-based containers from 9 to 20 days. Changing the sanitizing agent to peroxyacetic acid increased milk shelf-life to 34 days.

Simon and Hansen (2001b) pasteurized 2% milk at 92.2, 84.0 and 76.4ºC packaged in a variety of paperboard based containers and monitored its microbial load (SPC) for a period of four weeks. Milk processed at 76.4ºC had the lowest bacterial growth rate while milk processed at 84.0ºC had the highest bacterial growth rate. Milk samples stored at 1.7ºC maintained a lower SPC than those stored at 6.7ºC.

### 5.3.2 Effect of light

The effect of light on the development of off-flavors in milk has been repeatedly documented over time (Hoskin 1988, Bosset *et al.* 1993, Rysstad *et al.* 1998,

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

Skibsted 2000, Valero *et al.* 2000, Borle *et al.* 2001, Moyssiadi *et al.* 2004, Zygoura *et al.* 2004, Papachristou *et al.* 2006b). Both sunlight and to a lesser extent artificial light contribute to deterioration reactions resulting in off-flavor development in milk. Typical off-flavors encountered in milk include 'sunlight' or 'activated flavor' (cabbage, burnt feathers), which develops rather quickly, and 'oxidation flavor' (cardboard, tallowy, fallows), which develops more slowly. The first is due to the formation of methional formed through the Strecker degradation of the amino acid methionine (Marsili 1999). Sulfur compounds, such as sulfides and disulfides, also contribute to this type of off-flavor development. Oxidized flavor is a result of oxidation of unsaturated fatty acids under the influence of both oxygen and light. Numerous studies have shown that riboflavin acts as a photo-sensitizer for the development of activated flavors (Skibsted 2000, Rysstad and Kolstad 2006). Ascorbic acid is also involved in the process of off-flavor development. Dehydroascorbic acid (oxidized form of ascorbic acid) promotes oxidized flavor development, acting as a catalyst for the oxidation process. In the absence of oxygen ascorbic acid does not oxidize in the presence of light (Mottar 1985). Exposure to light also results in compositional changes of milk such as water-soluble (B2, B6, B12, C) and fat-soluble (A, E) vitamin degradation (Mottar 1985, Papachristou *et al.* 2006a,b). Finally, phodegradation may cause destruction of amino acids including methionine, histidine, tyrosine, tryptophan, cysteine and lysine (Mottar 1985). Harmful wavelengths responsible for off-flavor generation are those smaller than 500 nm.

Display cabinets in supermarkets have a mean light intensity of 1000 lux, which, despite being lower than daylight, may affect milk as soon as within 24 h, initiating off-flavor development. Most transparent packaging materials offer inadequate protection against harmful light wavelengths. Riboflavin destruction by light has been shown to be higher in skimmed milk than in whole milk, since harmful wavelengths (400–500 nm) can penetrate deeper into skimmed milk, due to the absence of fat, than into whole milk (Robertson 1993, Cladman *et al.* 1998). In a study investigating losses of vitamin A in skimmed milk stored under fluorescent light (intensity 2000 lumens/$m^2$ for 24 h) more than 75% of vitamin A was destroyed in glass, clear PC and PE bottles, while paperboard containers provided the best protection (Senyk and Shipe 1981). In another study by Mestdagh *et al.* (2005) on UHT semi-skimmed milk packaged in (a) PET with complete light barrier, (b) an active oxygen-binding inner layer, and (c) a UV-absorber stored under continuous illumination, it was shown that of the three containers only that with adequate light barrier protected the product from light-induced oxidation during extended storage. Likewise Van Aardt *et al.* (2001) showed that an amber-colored PET performed better than glass, HDPE and clear PET in preserving fresh milk flavor.

Wavelengths higher than 340 nm pass through uncolored glass and transparent and opaque plastic materials such as PE, PS and PET. Colored packaging, i.e. white, is capable of blocking harmful wavelengths up to 500 nm. The protection provided by colors follows the sequence black → brown → green

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

→ blue → red → yellow → uncolored. Black, being the most efficient, is used as a middle 'buried' layer in three-layer coextruded HDPE bottles for ultra-pasteurized milk packaging applications in which the expected shelf-life of the product is 15–20 days. Adequate protection from light and oxygen is provided by using paperboard PE packaging materials with an intermediate layer of aluminum foil in demanding applications such as UHT milk packaging. The necessity to protect milk from light extends also to milk powders which are sensitive to light-induced oxidation.

### 5.3.3 Effect of oxygen

As stated above, oxygen in combination with light or alone, as in the case of a large headspace within a container or as a result of permeation through the container walls, will cause fat oxidation, resulting in off-flavor development. When filling, pasteurized milk is saturated with oxygen (8–9 ppm). This concentration usually falls, being used up by aerobic spoilage microorganisms such as the Pseudomonads. Within the normal shelf-life of pasteurized milk this does not cause adverse reactions, given of course that no additional oxygen enters the container as would be the case with oxygen permeable packaging materials. Glass, being totally impermeable to oxygen, protects the product from additional oxygen gain through permeation (Schroeder 1982). Other commercial packaging materials such as polyethylene-coated paper board and PET also do not adversely affect pasteurized and ultrapasteurized milk and only in long-life products such as UHT milk is additional protection from oxygen and light achieved by the use of aluminum foil as an intermediate layer of the container. Gliguem and Birlouez-Aragon (2005) monitored vitamin C content of fortified milk samples (growth milks) using a three-layer opaque and a six-layer opaque HDPE bottle, the latter container bearing an ethylene vinyl alcohol (EVOH) oxygen barrier. Milk samples were stored at room temperature for four months in the dark. The use of the three-layer HDPE bottle was associated with complete oxidation of vitamin C after one month of storage; whereas in the six-layer barrier bottle, the vitamin C content slowly decreased to reach 25% of initial concentration after four months of storage.

IP Address: 129.132.208.100

### 5.3.4 Effect of water vapor permeability

Water vapor permeability of the packaging material is practically important only in milk powder products. When selecting a packaging material for such products the following factors should be considered: (1) initial moisture content of the powder, (2) final acceptable moisture content of the powder, and (3) shelf-life required. It is obvious that in order to achieve a maximum product shelf-life the moisture content or the water activity ($a_w$) should correspond to the minimal rate of lipid oxidation. It has been shown (Robertson 1993) that for whole powdered milk good flavor and oxidative stability were achieved at moisture content 3.5% ($a_w = 0.24$). With this in mind one can calculate the maximum amount of

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

moisture allowed to enter the package and propose a suitable packaging material based on its water vapor permeability. Commercial packaging materials for milk powders include three-piece metal cans in combination with $N_2$ flushing, pouch laminates consisting of aluminum foil 9 $\mu$m thick, paper of weight 45 g/m$^2$ and LDPE 25 $\mu$m thick. Alternatively a pouch made of PET 17 $\mu$m thick/LDPE 9 $\mu$m/aluminum foil 9 $\mu$m/LDPE 70 $\mu$m may be used. Fiber cans may also successfully be used, typically made of 0.9 mm board, aluminum foil coating 5 $\mu$m thick with a nitrocellulose lacquer to protect the powder from the aluminum foil (Cummins 1982).

### 5.3.5 Permeability to flavors

Package permeability to flavors is in principle similar to permeation to gases and is governed by the same physical laws. Transfer of odorous substances through the package can take place either from the environment into the packaged product or vice versa. In the first case, environmental contaminants penetrate the package walls and usually cause off-flavor development, while in the second case desirable flavor components of the products are lost to the environment through the package, causing reduction in flavor intensity of the packaged product. In the absence of micropores in the packaging material, permeation of volatile compounds proceeds through the mechanism of activated diffusion governed by Fick's law. In the presence of micropores, i.e. a poor closure, there is a simple mass flux of volatiles causing rapid flavor changes in the product. For products such as milk having only a bland characteristic aroma, loss of milk flavor through permeation of milk volatiles to the environment is of no practical importance. In contrast, since milk will easily absorb foreign flavors it is critical to select packaging materials with low permeability to flavor compounds. Dried milk products also easily absorb foreign odors due to the large surface area of the particles. Glass, metals and polyesters are highly impermeable to flavors while PE-coated paperboard is highly permeable. In applications involving extended shelf-life products, e.g. UHT milk, laminates, including aluminum foil, which is an excellent barrier to flavors, are used.

IP Address: 129.132.208.100

## 5.4 Migration and flavor scalping

Migration is the transfer of soluble or volatile components of the packaging material to the contained products. Conversely, flavor scalping is the transfer of soluble or volatile components of the contained product into the packaging material.

### 5.4.1 Migration

A large number of substances, mainly of low molecular weight, are capable of migrating from the packaging material into the contained product, often

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

affecting the sensory properties and occasionally product safety. Such substances include: residual monomers, processing additives such as plasticizers, stabilizers, antioxidants, antistatic agents, antiblocking agents, printing inks, etc. National (e.g. US FDA) and international (e.g. EU) legislation has set restrictions with regard to the upper acceptable limit of global migration (60 mg/kg of foodstuff or 10 mg/dm$^2$ of packaging material) (EEC Council Directive 90/128/EEC, EU Directive 2002/72/EC), and for numerous individual compounds a specific migration limit (e.g. 18 mg/kg for the plasticizer diethylhexyl adipate) (EU Synoptic Document No. 7) has been set based on the toxicity of various migrating species.

As mentioned previously, besides the critical aspect of food safety, deterioration of sensory properties of foodstuffs as a result of migration from the packaging material is another important issue. Typical examples of such a phenomenon include the transfer of styrene monomer from polystyrene containers into skimmed milk having a perception threshold of 0.3 mg styrene/liter and whole milk (3.7% fat) having a perception threshold of 1.2 mg styrene/liter (Hauschild and Spingler 1988). Another typical example is off-flavor development in porous snacks (cheese-flavored) due to the migration of residual organic solvents, e.g. ethyl acetate used as solvent for adhesives during the manufacture of laminated flexible food packaging materials (unpublished data, pers. comm. 2004).

Other examples of migration include determination of nonyl phenol (NP), a compound used as an antioxidant and plasticizer in HDPE and PVC, respectively, in milk surrogate (180–300 ng/l) (Loyo-Rosales *et al.* 2004) and of naphthalene in sterilized milk packaged in LDPE bottles (10–30 ng/ml) (Lau *et al.* 1994). Factors affecting extent of migration include the following:

- Type and amount of migrating species: properties such as polarity, hydrophobicity, size, etc., play a key role in migration.
- Properties and composition of foodstuffs: usually migration increases as viscosity decreases from dry to liquid foods. Migration also increases depending, of course, on the nature of the migrating species from aqueous to acidic and alcoholic products to fatty foodstuffs. Migration also increases with thickness of the packaging material.
- Time of contact: migration through the mechanism of diffusion has been shown to increase either with time or with the square root of time (Kondyli *et al.* 1992).
- Temperature: increasing temperature accelerates migration. However, temperature dependence does not follow the laws of diffusion, and extrapolation from test conditions to real-life practical conditions is not possible (IDF 1995).

Given the complex nature of most foods and the technical problems associated with determination of migrants in foodstuffs, the EU has approved a set of official food stimulants including (1) dimineralized water, (2) 3% aqueous acetic acid, (3) 15% aqueous ethanol, and (4) rectified olive oil, sunflower oil or a mixture of synthetic triglycerides (HB 307) to be used for migration testing. Test conditions are given in Table 5.1 (EEC Council Directive 82/711/EC, 1982).

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

**Table 5.1** Standard test conditions for the determination of substances migrating from polymer packaging materials into foodstuffs

| Conditions of practical use | Test conditions |
|---|---|
| Time of contact $t > 24$ h | |
| $T \leq 5$ºC | 10 days at 5ºC |
| 5ºC $< T \leq 40$ºC[a] | 10 days at 40ºC |
| Time of contact 2 h $\leq t \leq 24$ h | |
| $T \leq 5$ºC | 24 h at 5ºC |
| 5ºC $< T \leq 40$ºC[a] | 24 h at 40ºC |
| $T > 40$ºC | According to national regulation |
| Time of contact $t < 2$ h | |
| $T \leq 5$ºC | 2 h at 5ºC |
| 5ºC $< T \leq 40$ºC[a] | 2 h at 40ºC |
| 40ºC $< T \leq 70$ºC[a] | 2 h at 70ºC |
| 70ºC $< T \leq 100$ºC[a] | 1 h at 100ºC |
| 100ºC $< T \leq 121$ºC[a] | 30 min at 121ºC |
| $T > 121$ºC | According to national regulation |

[a] Test conditions of 10 days at 20ºC are also allowed for the analysis of polymer packaging materials entering into contact with food products that must be stored at temperatures below 20ºC according to inscription on the package or legal regulation.

### 5.4.2 Flavor scalping

IP Address: 129.132.208.100

Another source of flavor deterioration of the packaged product is flavor scalping. The phenomenon is more common in UHT processed milk, an application in which the product has a long shelf-life. It has been shown that flavor compounds such as aldehydes and ketones are selectively absorbed by LDPE and PP films altering the aroma of processed milk. PP showed a greater absorptive capacity than LDPE. Absorption of high molecular weight volatiles was higher after 12 weeks of storage in UHT milk. Besides PP and LDPE, PET has been shown to absorb volatiles of various foods, such as fruit juices, etc. (Hansen and Arora, 1990). Van Willige (2002) studied flavor scalping of LLDPE, PP, PC, PET and PEN by immersing strips of these plastics into a model flavor solution, corresponding to the flavor of whole and skimmed milk, at different temperatures for up to 14 days. He reported total absorption of flavor components to be 2400 times higher for LLDPE and PP than for PC, PET and PEN.

## 5.5 Environmental issues regarding packaging materials

Given the recent drastic increase in domestic waste around the world along with the need to protect the environment, a key issue for the packaging industry when introducing a new packaging system for a given application is the assessment of the impact the proposed package may have on the environment. Governments are running programs to reduce the amount of waste produced. Germany, with

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

its packaging ordinance of 12 June 1991 regarding the reduction in packaging waste, created a strong driving force for recycling and reuse of packaging materials. It is worth mentioning that in Germany 10% by weight (bw) of all domestic waste is composed of packaging materials.

The ordinance, effective 1 January 1993, states that 40% of all tinplate packaging, 60% of all glass packaging, and 30% of all aluminum, cardboard, paper and plastic packaging must be recovered; while from 1 July 1995 the recovery value for all packaging materials must rise to 80%. Thus, the Duales system of packaging waste management was created. Materials initially pre-sorted by consumers–users are collected and sorted by private and municipal waste management firms. They are then forwarded to recycling companies for processing and production of new packages or products. The collection of packaging waste is covered by contracts between Duales System Deutschland GmbH and municipal or private waste disposal firms. Following Germany, the EU with its Directives (94/62/EC, 2004/12/EC) on packaging and packaging waste aimed to align all member countries within the same packaging waste law. According to these EU Directives, all EU member states must adopt packaging recovery systems in order to achieve the following goals:

- Recovery or incineration for the production of energy of at least 50% bw of all packaging waste by 30 June 2001
- Recovery or incineration for the production of energy of at least 60% bw of all packaging waste by 31 December 2008
- Recycling of 25–45% bw of all packaging materials in municipal waste (at least 45% for each packaging material) by 30 June 2001
- Recycling of 55–80% bw of all packaging materials in municipal waste by 31 December 2008
- Recycling goals for individual packaging materials include 60% bw for glass, paper and paperboard, 50% bw for metals, 22.5% bw for plastics and 15% bw for wood (by 31 December 2008).

IP Address: 129.132.208.100

To compare packaging systems it is not enough to consider waste disposal aspects but to assess in detail the total life cycle (LC) of a given system. The LC of a packaging system covers all steps from packaging material manufacture to its use and its disposal/recovery. Thus, important information is gathered on energy consumption and pollution potential of each step of the LC. Environmental impact considers (1) the use of raw materials, particularly non-renewable resources, (2) energy consumption, (3) airborne and waterborne emissions, and (4) waste disposal. The specific packaging system to be adopted should rank low on all four considerations.

### 5.5.1 Reuse–multiple trip containers

Of the primary packaging materials the returnable milk bottle is the most typical example of the reuse principle. The effective cost of the returnable bottle is a function of its ability to withstand breakage and the effectiveness of the bottle

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

recovery system. Both factors are expressed in terms of bottle trippage. Returnable milk bottles are made of thicker glass than single-trip glass bottles to achieve a trippage equal to 30. The basic features of this system are collection of empties, washing and refilling. Operation times and capacities require storage rooms for both unwashed empties and washed bottles to be filled. For pasteurized milk, storage rooms must be refrigerated. Secondary packaging materials reused include plastic crates, metal and fiber drums, etc. In a study by Dovers *et al.* (1993) in Australia, environmental impacts associated with milk containers were reviewed. It was found that the traditional reuse glass bottle with a trip rate between 25 and 30 was judged the least environmentally damaging. The single-use (recyclable) glass bottle was judged the worst option environmentally. HDPE bottles and paperboard cartons as well as brick-type containers lay in between.

### 5.5.2 Recycling

*Aluminum*

Aluminum is ideal for recycling since the energy needed to produce a new can is only 10% of that needed when starting from primary resources (Mondorf and Jensen 1995).

*Tinplate*

For tinplate the respective amount of energy to make a new can from an old one is close to 40% as compared to that when starting from iron ore. Sorting involves (1) presorting from other waste materials in households and (2) magnetic post-sorting from shredded household waste. The most suitable sorting method will depend on population trends, i.e. large cities vs. sparsely populated areas.

*Glass*

Appreciable amounts of energy are consumed in the melting furnace for the manufacture of glass from fusion of soda ash, sand and limestone. The energy required to remelt glass after recovery of waste glass is considerably lower. Usually colored glass is collected separately from colorless glass to achieve better economy during processing.

*Plastics and paper*

As opposed to metals and glass, plastics and paper can be used as fuel for energy production by incineration. For polyethylene, which makes up more than 50% bw of commercial plastics, it takes approximately 15–25% of the energy required for manufacturing the same quantity of PE products from recycled PE as when one starts from virgin raw materials. Multiple recycling cycles are recommended and when this is no longer possible incineration or pyrolysis may be used, recovering approximately 70% of the energy content of PE (Mondorf and Jensen 1995).

PE, PVC and PET can be recycled very successfully. It is, however, difficult to recycle multilayer barrier plastics. According to Tetrapak, multilayer

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

containers are commingled with wood products and used to produce 'plasticized wood' products such as fences, patio tables, chairs, etc. In Scandinavia empty multilayer milk cartons are collected, the paperboard is dissolved and processed into new paper products (e.g. egg boxes), while the PE and aluminum layers are separated and reused for different purposes.

Mourad *et al.* (2008) applied life cycle assessment (LCA) to 1000 liters of milk packaged in Tetrapak Aseptic containers and showed (for cardboard recycling only) a 14% reduction in global warming potential (GWP) when increasing the recycling rate from 2% (2000) to 22% (2004). It was calculated that a 48% reduction in GWP would result when the recycling rate reaches 70%. Welle (2005) investigated the recycling process of HDPE milk bottles involving an efficient sorting process, hot washing of ground bottles and a further deep-cleaning of the flakes at high temperatures under vacuum. Results showed that the particular recycling process was suitable for recycled post-consumer HDPE bottles for direct food-contact applications.

For paper the situation is similar to that for PE. Recycling of paper/paperboard results in roughly a 35% energy saving, while a 25% saving is achieved through incineration producing useful heat.

### 5.5.3 Incineration and sanitary landfilling

Incineration is an acceptable second alternative when recycling is not possible. Incineration results in a large reduction in waste volume while solid residue is produced as a sterile mass. Magnetic metals are removed from waste before incineration, while other metals are sorted from the ash produced as a product of incineration. The greatest advantage of incineration is, however, the possibility of using the energy of combustion as heat energy. It has been estimated that the calorific value of domestic waste is *ca.* 3000 kcal/kg while that of wood is 4000 kcal/kg and that of fuel oil is 12,000 kcal/kg. Plastics being produced from fuel oil rank particularly high in calorific value. The main disadvantage of incineration is environmental pollution. Among packaging materials, vinyl derivatives (PVC, P(VDC-VC)) produce a large amount of hydrogen chloride, a highly corrosive gas, when incinerated. Dioxins are also produced by burning paper and plastics (Mondorf and Jensen 1995). To solve this serious problem numerous companies have developed specially designed incineration and pyrolysis municipal waste systems to trap all harmful gaseous products produced.

After application of the principles of (1) reduction of the amount of packaging material at source, (2) reuse–recycling and (3) incineration, the last alternative for waste disposal is sanitary landfilling (SLF). In SLF, waste is deposited as layers sandwiched between layers of soil in large areas insulated from the environment using synthetic geo-membranes to protect groundwater contamination. Under such conditions of waste disposal, microorganisms rapidly use up any oxygen present and thus decomposition of organic material becomes anaerobic, causing formation of gases, mainly methane. Plastics and even paper are not decomposed in a SLF in the absence of oxygen, light and moisture, and

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

such materials have been found intact in landfills after many years. Given the ever-increasing amounts of domestic waste produced around the world and the shortage of available space, scientists are exploring turning to methods of waste treatment such as composting, thermal gasification, pyrolysis, etc. (Taralas *et al.* 2003).

## 5.6 Sources of further information and advice

BRODY AL and MARSH KS (1996), *The Wiley Encyclopedia of Packaging Technology*, 2nd edn, Wiley Interscience, New York.

EU Directive 94/62/EC of the European Parliament and of the Council on Packaging and Packaging Waste, 20 December 1994, Brussels.

FAO (2007), *Packaging, Storage and Distribution of Processed Milk*, Chapter 2, http://www.fao.org/DOCREP/003/X6511E/X6511E02.htm

IDF (1995), *Bulletin of the International Dairy Federation*, No. 300, Brussels, Chapters 3 (pp. 8–11), 4 (pp. 12–16), 11 (pp. 65–70), 13 (pp. 78–83), 16 (pp. 95–97) and 17 (pp. 98–100).

JENKINS A and HARRINGTON JP (1991), *Packaging Foods with Plastics*, Technomic Publishing Co., Lancaster, PA, Chapter 11, pp. 145–171.

PAINE FA (1996) *The Packaging User's Handbook*, Blackie Academic and Professional, London, Chapters 3 (pp, 36–63), 4 (pp. 64–80), 5 (pp. 81–93), 6 (pp. 99–101) and 7 (pp. 102–120).

PAINE FA and PAINE HY (1992), *Handbook of Food Packaging*, 2nd edn, Blackie Academic and Professional, London, Chapter 7, pp. 205–230.

ROBERTSON GL (1993), *Food Packaging: Principles and Practice*, Marcel Dekker, New York, Chapter 17, pp. 507–549.

THE DAIRY COUNCIL (2007), *Varieties of Milk*, 19 December 2007 http://www.milk.co.uk/page.aspx?intPageID=43.

US-FDA (2003), Grade 'A' Pasteurized Milk Ordinance, CF SAN/Office of Compliance, 2 March, 2004.

IP Address: 129.132.208.100

## 5.7 References

BORLE F, SIEBER R and BOSSET LO (2001), Photooxidation and photo-protection of foods with particular reference to dairy products. An update of a review article (1993–2000), *Sciences des Aliments* 21(6), 576–590.

BOSSET JO, GALLMANN PU and SIEBER R (1993), Influence of light transmittance of packaging materials in the shelf life of milk and dairy product, a review, *Lait* 73(1), 3–49.

BRODY AL and MARSH KS (1996), *The Wiley Encyclopedia of Packaging Technology*, 2nd edn, Wiley Interscience, New York.

CERF D and CONDRON P (2006), *Coxiella burnetii* and milk pasteurization: an early application of the precautionary principle? *Epidemiol. Infect.* 134, 946–951.

CLADMAN W, SCHEFFER S, GOODRICH N and GRIFFITHS MW (1998) Shelflife of milk packaged in plastic containers with and without treatment to reduce light transmission, *Int. Dairy J.* 8, 629–636.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

CUMMINS N (1982), Milk powders, in *Technical Guide to the Packaging of Milk and Milk Products*, No. 143, 2nd edn, IDF, Brussels, Chapter 19.

DEMERTZIS PG, JOHANSSON F, LIEVENS C and FRANZ R (1997), Studies on the development of a quick inertness test procedure for multi-use PET containers – sorption behavior of bottle wall strips, *Packaging Technology and Science* 10, 45–58.

DIMMICK B (2007), Packing punch with packaging, *Milk Producer*, 26 June, 32.

DOVERS S, MADDEN E, COMMON M and BOYDEN S (1993), Milk packaging in Australia: a case study in environmental priorities, *Resources, Conservation and Recycling* 9(1–2), 61–73.

EEC Council Directive 82/791/EEC, 18 October 1982, Brussels.

EEC Council Directive 90/128/EEC Relating to Plastic Materials and Articles Intended to Come into Contact with Foodstuffs, 23 February 1990, Brussels.

EU Directive 94/62/EC of the European Parliament and of the Council on Packaging and Packaging Waste, 20 December 1994, Brussels.

EU Directive 2002/72/EC Relating to Plastic Materials and Articles Intended to Come into Contact with Foodstuffs, 6 August 2002, Brussels.

EU Directive 2004/12/EC of the European Parliament and of the Council on Packaging and Packaging Waste, 11 February 2004, Brussels.

EU Synoptic Document No. 7, Draft of Provisional List of Monomers and Additives Used in the Manufacture of Plastics and Coatings Intended to Come into Contact with Foodstuffs, 1994, Brussels.

FALLA DJ (2004), Using enhanced polyolefin technology in pouches for packaging flowable materials, *Engineering Plastics* 9(5), 384–402.

FAO (2007), *Packaging, Storage and Distribution of Processed Milk*, Chapter 2, http://www.fao.org/DOCREP/003/X6511E/X6511E02.htm

FARRER KTH (1983), *Light Damage in Milk*, Farrer Consultants, Blackburn, Victoria 3130, Australia.

GLIGUEM H and BIRLOUEZ-ARAGON I (2005), Effects of sterilization packaging and storage on vitamin C degradation, protein denaturation and glycation in fortified milks, *J. Dairy Sci.* 88, 891–899.

GRØNBORG H (1995), in *Bulletin of the International Dairy Federation*, No. 300, IDF, Brussels, Chapter 11.

GRUETZMACHER IJ and BRADLEY JR. RL (1999), Identification and control of processing variables that affect the quality and safety of fluid milk, *J. Food Prot.* 62(6), 625–631.

HANSEN AP and ARORA DKC (1990), Loss of flavor compounds from aseptically processed food products packaged in aseptic cartons, in *Barrier Polymers and Structures*, WI Koros (ed.), ACS Symposium Series No. 423, ACS, Washington, DC, Chapter 17.

HAUSCHILD G and SPINGLER E (1988), *Migration bei Kunstoff-verpackungen*, Wissenschaftliche Verlagsgesellschaft, Stuttgart.

HOSKIN JC (1988), Effect of fluorescent light on flavor and riboflavin content of milk held in modified half-gallon containers, *J. Food Prot.* 51(1), 19–23.

IDF (1995), *Bulletin of the International Dairy Federation*, No. 300, IDF, Brussels, Chapter 3.

INEOS POLYOLEFINS (2007), http://www.ineospolyolefins.com

KARATAPANIS AE, BADEKA AV, RIGANAKOS KA, SAVVAIDIS IN and KONTOMINAS MG (2006), Changes in flavor volatiles of whole pasteurized milk as affected by packaging material and storage time, *Int. Dairy J.* 16, 750–761.

KONDYLI E, DEMERTZIS PG and KONTOMINAS MG (1992), Migration of dioctylphthalate and

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

dioctyladipate plasticizers from food-grade PVC films into ground-meat products, *Food Chem.* 45, 163–168.

LANDSBERG JD, BODYFELT FW and MORGAN ME (1977), Retention of chemical contaminants by glass, polyethylene, and polycarbonate multiuse milk containers, *J. Food Prot.* 40, 772–777.

LAU OW, WONG SK and LEUNG KS (1994), Naphthalene contamination of sterilized milk drinks contained in low density polyethylene bottles, part I, *Analyst* 119(5), 1037–1042.

LOYO-ROSALES JE, ROSALES-RIVERA GC, LYNCH AU, RICE CP and TORRENS H (2004), Migration of nonyl phenol from plastic containers to water and a milk surrogate, *J. Agric. Food Chem.* 52(7), 2016–2020.

MARSILI RT (1999), Comparison of SPME and dynamic headspace method for the GC/MS analysis of light-induced lipid oxidation products in milk, *J. Chromatogr. Sci.* 37, 17–23.

MESTDAGH F, DE MEULENAER B, DE CLIPPELEER J, DEVLIEGHERE F and HUYGHEBAERT A (2005), Protective influence of several packaging materials on light oxidation of milk, *J. Dairy Sci.* 88, 499–510.

MONDORF U and JENSEN F (1995), in *Bulletin of the International Dairy Federation*, No. 300, IDF, Brussels, Chapter 13.

MOTTAR J (1985), in *Bulletin of the International Dairy Federation*, No. 143, IDF, Brussels, Chapter 8.

MOTTAR J (1987), The usefulness of co-extruded high density polyethylene for packaging UHT milk, *IDF Dairy Packaging Newsletter*, No. 15, IDF, Brussels.

MOURAD AL, GARCIA EEC, VILELA GB and VON ZUBEN F (2008), Influence of recycling rate increase of aseptic cartons for long-life milk on GWP reduction, *Resources, Conservation and Recycling* 52(4), 678–689.

MOYSSIADI T, BADEKA A, KONDYLI E, VAKIRTZI T, SAVVAIDIS I and KONTOMINAS MG (2004), Effect of light transmittance and oxygen permeability of various packaging materials on keeping quality of low-fat pasteurized milk: Chemical and sensorial aspects, *Int Dairy J.* 14, 429–436.

PAINE FA (1996), *The Packaging User's Handbook*, Blackie Academic and Professional, London, Chapter 1.

PAPACHRISTOU C, BADEKA A, CHOULIARA E, KONDYLI E, ATHANASOULAS A and KONTOMINAS MG (2006a), Evaluation of PET as a packaging material for premium quality whole pasteurized milk in Greece, Part I, *Eur. Food Res. Technol.* 223, 711–718.

PAPACHRISTOU C, BADEKA A, CHOULIARA E, KONDYLI E, KOURTIS L and KONTOMINAS MG (2006b), Evaluation of PET as a packaging material for premium quality whole pasteurized milk in Greece, Part II, *Eur. Food Res. Technol.* 224, 237–247.

ROBERTSON G (1993), *Food Packaging: Principles and Practice*, Marcel Dekker, New York, Chapter 17.

RYSSTAD G and KOLSTAD J (2006), Extended shelf life milk: advances in technology, *Int. J. Dairy Technol.* 59(2), 85–96.

RYSSTAD G, EBBESEY A and EGGESTAD J (1998), Sensory and chemical quality of UHT milk stored in paperboard cartons with different oxygen and light barriers, *Food Addit. Contam.* 15(1), 112–122.

SATTAR A and DEMAN JM (1973), Effect of packaging material on light induced quality deterioration of milk, *J. Can. Inst. Food Sci. Technol.* 6, 170–174.

SCHROEDER MJA (1982), Effect of oxygen on the keeping quality of milk, *J. Dairy Res.* 49, 407–424.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

SENYK GF and SHIPE WF (1981), Protecting your milk from nutrient losses, *Dairy Field* 164(3), 81–85.

SIMON M and HANSEN HP (2001a), Effect of various dairy packaging materials on the shelf life and flavor of ultrapasteurized milk, *J. Dairy Sci.* 64, 784–791.

SIMON M and HANSEN AP (2001b), Effect of various dairy packaging materials on the shelf life and flavor of pasteurized milk, *J Dairy Sci.* 84(4), 767–773.

SKIBSTED LH (2000), Light induced changes in dairy products, *Bulletin of the International Dairy Federation*, No. 345, IDF, Brussels.

TARALAS G, KONTOMINAS MG and KAKATSIOS X (2003), Modeling thermal destruction of toluene as tar-related species for fuel gas clean-up, *Energy and Fuels* 17, 329–337.

VALERO E, VILLAMIET M, SANZ J and MARTINEZ-CASTRO J (2000), Chemical and sensorial changes in milk quality on the keeping quality of pasteurized milk, *Lett. Appl. Microbiol.* 20, 164–167.

VAN AARDT M, DUNCAN SE, MARCY JE, LONG TE and HACKEY CR (2001), Effectiveness of PET and HDPE in protection of milk flavor, *J. Dairy Sci.* 84, 1341–1347.

VAN WILLIGE RWG (2002), *Effects of flavor absorption on foods and then packaging materials*, PhD thesis, Wageningen University, the Netherlands.

VASSILA E, BADEKA A, KONDYLI E, SAVAIDIS I and KONTOMINAS MG (2002) Chemical and microbiological changes in fluid milk as affected by packaging conditions, *Int. Dairy J.* 12, 715–722.

WELLE F (2005), Post-consumer contamination in high-density polyethylene (HDPE) milk bottles and the design of a bottle-to-bottle recycling process, *Food Additives and Contaminants* 22(10), 999–1011.

ZYGOURA P, MOYSSIADI T, BADEKA A, KONDYLI E, SAVVAIDIS I and KONTOMINAS MG (2004), Shelf life of whole pasteurized milk in Greece: effect of packaging material, *Food Chem.* 87, 1–9.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

# 6

# Sensory evaluation of milk

**K. W. Chapman, Cornell University, USA**

**Abstract:** Powerful results will be achieved when using appropriate sensory tests in the accurate manner, with proper analysis and interpretation of results. This chapter reviews common methods used in the sensory evaluation of milk such as discrimination tests, descriptive analysis, acceptance (hedonistic), preference and threshold testing.

**Key words:** sensory evaluation, milk, dairy, descriptive analysis, discrimination, acceptance, preference, threshold.

IP Address: 129.132.208.100

## 6.1 Introduction: key issues in the sensory evaluation of milk

Quality of processed milk can be evaluated by trained scientists in a variety of ways, such as chemical and microbiological analyses. Consumers, however, will make their own analysis, based on their perceptions of whether this milk tastes good. Therefore, the ultimate evaluation is sensorial and is done by the consumer. Systematic sensory analysis is needed to provide useful information about the human perception of milk. Such sensory evaluation is key to the improvement of product quality and shelf-life, enabling milk to compete with innovative new introductions, as well as with currently popular shelf-stable products. Whenever new procedures are developed to reduce the microbiological growth and biochemical changes, sensory evaluation is important in determining whether these will meet with consumer acceptance. Sensory evaluation is also important for new product development, such as increasing the nutritional value of milk, e.g., by iron (Rodriguez, 2007) or folic acid (Achanta *et al.*, 2007) fortification, or formulating low-fat milk so that it tastes more like full-fat milk (Phillips and Barbano, 1997). From a consumer perspective,

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

sensory characteristics most directly influence product acceptability. If the milk does not taste good, there will not be repeat purchases.

This chapter will provide some historical perspective on human perceptual evaluations of milk and a description of common methods currently in use to conduct sensory evaluation. This is followed by discussion of the major sensory analysis tools employed, along with examples, and additional sources of information about sensory evaluation.

## 6.2 Historical perspective

In the latter part of the nineteenth century the grading of dairy products first received national and international attention. The establishment of product grades such as the American Dairy Science Association (ADSA) score cards, as well as standards for various dairy products, closely paralleled the growth of the dairy industry and development of dairy product markets (Bodyfelt *et al.*, 1988). The ADSA score cards have also been used by the International Collegiate Dairy Products Evaluation Contest since 1916 (Trout *et al.*, 1939). The score card is a tabulated list of the various factors used to assess the quality of dairy products. A numerical value is assigned to each factor (Bodyfelt, 1981).

The beginning of the twentieth century marked the establishment of major brands and trade names for dairy products. Some brands of dairy products have become widely known for their high quality. For instance, Sealtest, Inc. established the 'Sealtest brand' recognizing their high quality milk. Brand development necessitated recognition of set standards of finished products by an experienced, competent judge (Bodyfelt *et al.*, 1988).

IP Address: 129.132.208.100

American agricultural writers, from early on, recognized that the consumption of dairy products depended primarily upon their flavor characteristics. These writers cautioned dairymen concerning feeding and milk handling practices to obtain a high quality dairy product. For example, Deane (1797) advised: 'In feeding milch cows, the flavour of the milk should be attended to … Feeding them with turnips is said to give an ill taste to the butter made of the milk.'

During the last 25 years, sensory science has advanced tremendously (Goff and Griffiths, 2005; Barbano and Lynch, 2006). 'Sensory Science is enjoying a period of strong growth, both at the intellectual and at the practical levels' (Moskowitz *et al.*, 2003, p. 1). Sensory evaluation of milk has evolved from a defect identification oriented system involving the use of the score card by a single expert, the dairy judge, to an attribute intensity scaling approach that uses trained panelists for quantitatively describing many dimensions of a product's characteristics. Ten to 12 trained panelists evaluate the intensities of the attributes to profile the aroma, flavor, aftertaste, and texture of fluid milk (Stone and Sidel, 2004b). These specific test methods take the form of planned experiments.

The traditional dairy judging approach for evaluating fluid milk for sensory characteristics is based on scoring a product against a specified list of defects commonly found in conventionally pasteurized milk. Traditional dairy judging

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

has been criticized for failure to predict consumer acceptance, lack of objectivity in quality assessments, difficulty in assignment of quantitative scores, and lack of utility for combining analytically oriented attribute ratings with affectively oriented quality scores (Claassen and Lawless, 1992). In addition to these shortcomings, application of traditional judging strategies to new products, such as extended shelf-life ultra-pasteurized (UP) milk, raises further analytical challenges, as changes in UP product characteristics are more subtle and occur over a longer period of time than changes typically encountered in conventionally pasteurized milk (Boor and Nakimbugwe, 1998; Shipe, 1980). Thus, as the dairy industry moves toward production of extended shelf-life products, the need emerges for development of appropriate sensory tools that are sensitive and specific for these products.

## 6.3 Principles of sensory evaluation

Sensory evaluation has been defined as a scientific method used to evoke, measure, analyze, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste, and hearing (Stone and Sidel, 2004a). The best raw materials and ingredients yield the best products; hence, sensory quality is a crucial consideration for finished product ingredients such as fluid and dried milk. When dealing with dairy foods, sensory quality is always involved (Clark *et al.*, 2009).

The degree of liking is not the only question answered by sensory analysis. For example, trained panelists can be used to generate data that are objective and analogous to instrumental data; threshold tests can be used to estimate sensory thresholds and qualitative tests can be used to determine consumer emotional responses to products. Shelf-life studies can be used to determine how long a product will taste good, which is critical with dairy products, because of their fragile nature. Consumer perception, the impact of storage, ingredient substitution, packaging, and process variability can be quantified; and correlation can be established between instrumental tests and sensory perception. To meet a specific objective, the correct sensory test must be chosen.

IP Address: 129.132.208.100

## 6.4 Examples of evaluation methods, their application and effectiveness

Sensory evaluation comprises a set of techniques for product presentation and well-defined response tasks, statistical methods, and guidelines for interpretation of results. There are many tools in the sensory toolbox, but these tools basically fit into three broad categories, each with a different goal and using different criteria for panelist selection. These three primary kinds of sensory tests focus on the existence of overall differences among products (discrimination tests), specification of attributes (descriptive analysis), and measuring consumer likes

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

and dislikes (affective or hedonic testing). Once the test objective has been determined, an appropriate test method must be selected along with panel setup, experimental design, test setup, ballot decisions, and scale/question decisions. After the test is conducted, the results are analyzed and interpreted.

No matter which method is chosen, standard sensory practices should be used in order to assure consistent, actionable data (Lawless and Heymann, 1998). Good practices include a controlled sensory testing environment, test protocol considerations (sample serving procedures, sample size, sample serving temperature, serving containers, carriers, palate cleansing), experimental design, and panelist qualifications, as well as accurate tabulation and appropriate statistical analysis.

### 6.4.1 Discrimination tests

If the objective is to determine whether two samples are perceptibly different, discrimination tests should be used (Peryam, 1958; Amerine *et al.*, 1965; Meilgaard *et al.*, 2006; Stone and Sidel, 2004b). Product developers often need to reformulate a product by using different ingredients. They do not want the consumer to detect a difference, and it is possible for two samples to be chemically different in formulation but for humans not to perceive this difference. For example, a flavored milk manufacturer wants to substitute a less expensive vanilla extract in its chocolate milk. The objective would be to determine whether consumers can detect a difference between the control and the new chocolate milk. The triangle test is an example of a discrimination test that could be used for the above situation. In the triangle test, panelists are given three samples, two the same and one different. The panelist is asked to identify the different sample. The number of correct responses is recorded. Tables are used to determine if the results are statistically significant, i.e. not just due to chance performance or correct guessing. Discrimination testing can also be used if the manager wants to know whether, for instance, a new procedure for pasteurizing milk will be noticeable. There are many different discrimination tests. Some of these tests involve sorting different samples from a set of alternatives such as the triangle test. Others involve matching test samples to standards, such as the duo–trio, ABX and dual standard tests. Others involve choosing the weakest or strongest sample on a specified attribute such as the paired comparison or *n*-alternative forced choice test. All of these have the following in common: an answer is forced ('don't know' is not an option) and there is a chance guessing level of expected correct choices when there is no real difference. Straightforward statistical tests can then test against this chance level of correct choices.

Another type of discrimination test that is useful for the dairy industry in determining the minimum concentration of an off-flavor compound involves sensory threshold measurement. Several issues must be addressed to determine an accurate and reproducible threshold value. Thresholds are impacted by several factors, and perhaps the most significant element is a proper and

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

consistent testing procedure. This includes an appropriate threshold method, an appropriate number of panelists, and consistent methodology. Examples of threshold methods are the following: ascending forced-choice or 3-AFC method of limits, R-index method (signal detection) and CHARM analysis (yes/no response) (ASTM, 1992; Lawless and Heymann, 1998). With CHARM analysis a person sniffs the effluent of a gas chromatograph to separate volatile flavors and measure several thresholds at once.

An example of discrimination testing on milk is the Matak *et al.* (2007) study where triangle tests revealed differences between the odors of raw milk and ultraviolet (UV) irradiated milk. Triangle tests were also used by Prescott *et al.* (2005) to determine the detection threshold for 2,4,6-trichloroanisole (TCA) in wine. Cork taint in wine produced by TCA is characterized by generally unacceptable musty or earthy odors.

### 6.4.2 Descriptive analysis

Descriptive analysis consists of training a group (usually 6–12) of individuals to identify and quantify specific sensory attributes or all of the attributes of a food. The extent of the training is dependent upon the complexity of the sensory attributes that are to be profiled.

Descriptive analysis is ideal for shelf-life testing (the length of time during which a food product performs satisfactorily), especially if the panelists are well trained and available, and give consistent results over time. However, descriptive panels are not asked about likes and dislikes, i.e. they are used like an analytical instrument. The results can be correlated with chemical and/or microbiological analysis (Hedegaard *et al.*, 2006). These methods can also be used to determine where a niche lies in the prototype products on the market. In quality control, descriptive techniques can be invaluable when a problem must be defined. Some examples of these tests are Quantitative Descriptive Analysis (Stone and Sidel, 2004b), Spectrum, Flavor Profile, and Texture Profile (Meilgaard *et al.*, 2006).

IP Address: 129.132.208.100

### 6.4.3 Acceptance and preference tests

Acceptance (hedonic) and preference testing with consumers is usually performed toward the end of the product development process. When testing the preference of one product directly against a second product, the paired preference test can be used, while with more than two products, a preference ranking test can be used. The most commonly used scale to determine the degree of liking is the 9-point hedonic scale. The scale is anchored with words ranging from 'like extremely' to 'dislike extremely' as in Fig. 6.1 (Lawless and Heymann, 1998). There are similar scales for the testing of acceptance by children that use a smaller number of points with kid-friendly words or smiley faces (Kroll, 1990). Hedonic data can be used in preference mapping. This is a very valuable procedure that allows visualization of the directions of product preferences in spatial models of a product set.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

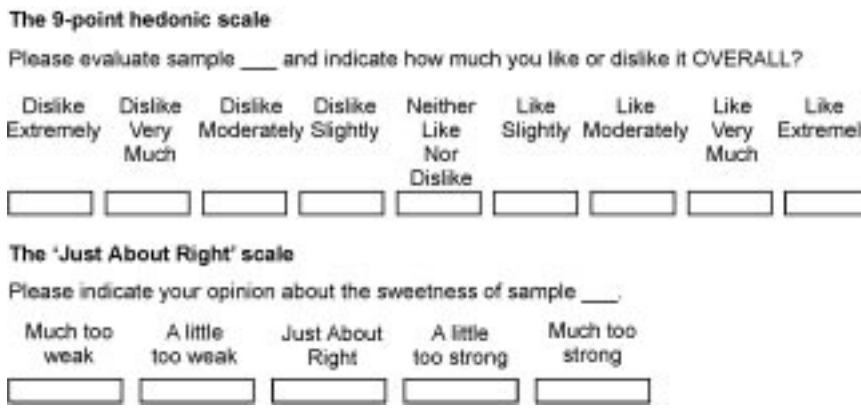



**Fig. 6.1** Examples of hedonic scales: 9-point hedonic scales (for measuring intensity of liking) and 'just about right' (JAR) for measuring desirability of a specific attribute.

Just Right scales are variations of acceptance testing. The Just Right scale, also called the Just About Right (JAR) scale, measures the desirability of a specific attribute and can be used to determine the optimum level of attributes. For example, in testing chocolate milk, the JAR scale can be anchored with 'not sweet enough' on the left, 'just about right' in the center, and 'much too sweet' on the right. Directional information for product reformulation or optimization can be gleaned from JAR scales, which combines intensity and acceptance judgments (Meilgaard *et al.*, 2006). The Labeled Affective Magnitude scale can be used for assessing food likes/dislikes. It has an increased ability to differentiate among extremely well liked or extremely disliked foods (Schutz and Cardello, 2000) (Fig. 6.2).

When new procedures are developed for processing milk, the most important question to answer is how much consumers like the taste of the milk. A study from the Cornell Sensory Testing Facility (Chapman *et al.*, 2001) utilized sensory evaluation procedures to measure children's acceptance of three types of milk. Children, 6 to 11 years of age, were asked hedonic questions about how they felt about milk in general and also their degree of liking. A 7-point facial hedonic scale with verbal descriptors for affective testing with children, anchored by 'Super bad' and 'Super good' (Kroll, 1990; Resurreccion, 1998) was used to evaluate three types of milk: milk conventionally pasteurized by high temperature short time (HTST), ultra-pasteurized milk (UP), and ultra-high temperature (UHT) milk. The children preferred HTST to UHT milk, which was preferred over UP milk. Children's opinions of the sensory quality of milk should be taken seriously. As UP milks are often distributed in fast food establishments, a place where children commonly drink milk, attention should be directed toward making these products more appealing. According to Dr Joseph Hotchkiss, packaging specialist from Cornell University, Ithaca, NY, 'If you want to increase milk sales, make sure the milk appeals to children. If they like milk when they were young, they are more apt to drink it as an adult' (personal communication, 1998).

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

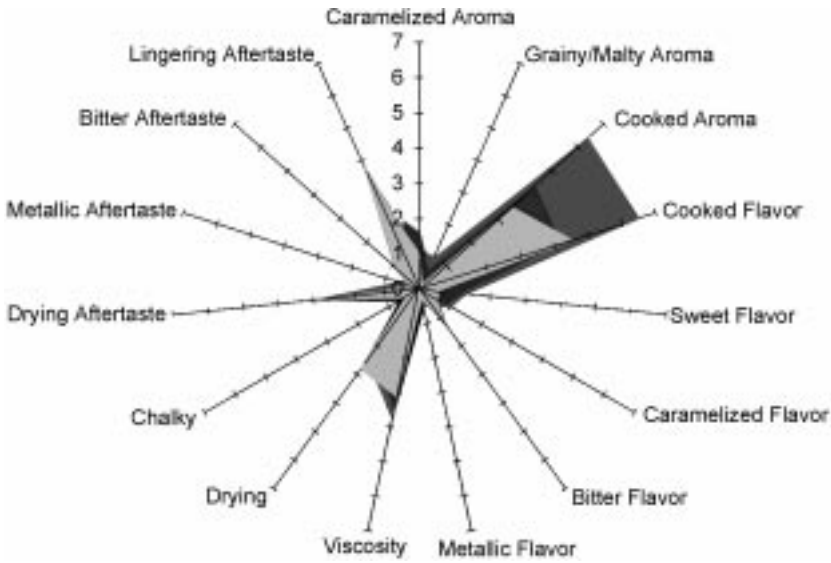



**Fig. 6.2** Sensory profiles of reduced fat milk stored at 6ºC. Individual attributes are positioned like the spokes of a wheel around a center (zero, or not detected) point, with the spokes representing attribute intensity scales, with higher (more intense) values radiating outward. Legend: dark gray area is day 2, black area is day 29, and light gray area is day 61. (Taken from Chapman *et al.*, 2001, with permission.)

IP Address: 129.132.208.100

Milk is a perishable product and has a relatively short shelf-life. Therefore, extending the shelf-life of milk will contribute to the competitiveness of the dairy industry in the beverage market. The shelf-life of milk can be influenced by many interacting factors, such as raw milk quality, processing conditions, storage temperature, oxygen pressure, light, and package configuration. Experiments have been designed to determine the shelf-life of milk, which consist of subjecting several samples of a product to tests and observing failure rates over time. Shelf-life testing of milk involves sensory testing for observing and tasting changes in the quality of the product. This may involve the length of time it takes for either the quality to deteriorate to the extent that the product has an off-flavor or until it becomes unfit for human consumption, depending upon the criterion agreed upon for judging the product's failure. Correlating consumer acceptability to trained panelists' data to define sensory failure is an improvement over more arbitrary criteria (Hough *et al.*, 2002).

The Milk Quality Improvement Program (MQIP) at Cornell University follows a strict protocol when performing shelf-life studies. The test objective is to determine the shelf-life of representative milk samples of dairy plants in and around New York State. Quantitative descriptive analysis was used to identify the attributes for the ultrapasturized-milk ballot. About twelve panelists are trained to reliably identify and rate product attributes and appropriate intensities with refresher training sessions given at least biannually. Six panelists are then used during each shelf-life study. At each testing period, sample containers are

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

mixed by inversion. Then, in dim light, 60 ml of sample are poured into 148 ml plastic cups (with blind three-digit codes) and capped with the appropriate plastic lid, and placed in a cardboard box. The box of samples is presented to panel members seated in individual booths when the samples reached 15ºC. Panelists evaluate the milk according to the following method:

1. Each cup is swirled and the odor is noted by removing the lid and placing the nose directly over the cup and sniffing the headspace.
2. The flavor is noted by taking a generous sip, rolling the milk around in the mouth and then expectorating.
3. After-taste is enhanced by drawing a breath of air slowly through the mouth and then exhaling slowly through the nose (Chapman *et al.*, 1998).

Panelists are provided water and unsalted saltine crackers for rinsing and palate cleansing. Each panelist uses an individual booth equipped with a computer that leads them through the tests and collects data using Compusense® *five* software (ver. 4.6; Guelph, ON, Canada). The ballot includes intensity rating scales for each attribute and an overall quality rating. The panelists perform independent rating observations on randomly ordered samples of milk. They only rate the attributes that are perceived in the milk sample (Fig. 6.3). When two or more panelists note an attribute, that attribute and its intensity are reported to the dairy plant.

As product quality drives consumer acceptance and demand, the ability to measure sensory attributes characteristic of high quality products is necessary for development and production of products that meet consumer expectations. Whited *et al.* (2002) used a trained panel to determine the effects of light exposure on vitamin A degradation and on light-oxidized flavor development of whole, reduced fat, and nonfat milk for 2 to 16 hours and found that even brief moderate light exposure can reduce the nutritional value and flavor quality of milk.

After a failure criterion has been agreed upon, the time to failure can be observed. If the criterion is the point in time at which observers first respond to a stimulus, threshold testing will have a key role in the shelf-life determination. Sensory evaluation has been successfully used to measure the threshold of odor or taste in milk. Thresholds are the limits of sensory capacities, the point at which a sensory signal becomes minimally detectable (Meilgaard *et al.*, 2006). For example, the semi-ascending paired difference method was used to evaluate the sensory threshold for changes in milk flavor resulting from oxidizing effects of light (Chapman *et al.*, 2002). Trained panelists were able to detect flavor defects following as little as 15 to 30 minutes of light exposure, while consumers detected defects between 54 minutes and 2 hours.

Since product quality drives consumer acceptance and demand, the ability to sensitively measure sensory attributes characteristic of high quality products is necessary for the development and production of products that meet consumer expectations. An ascending two-alternative forced choice test with 12 panelists was used, for example, to establish the threshold for certain dairy-related flavor

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

# Milk Quality Improvement Project Presents this Shelf-life Study on Ultra-Pasteurized Milk Box 1

**Panelist Code: _____**

A. Compare your samples with the references.
   1. Use the **2% Cornell milk** reference for '**cooked**' and '**2% fat**'
   2. Use the **100% lactose reduced** reference for **pronounced 'sweet**.'

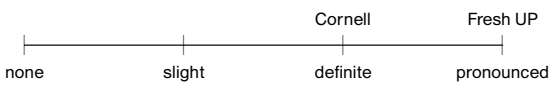
B. When rating the attributes, you may click anywhere on the intensity line.

C. Start with sample %01 on left.

D. You only have to rate those attributes that are relevant.

**Question #1 Please evaluate the following Aroma attributes for Sample __**

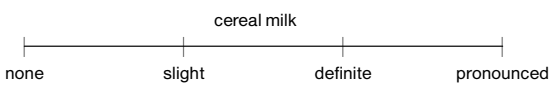
Cooked Aroma



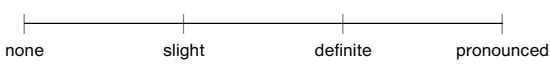

Caramelized Aroma




Grainy/malty aroma




Other Aroma




**Question #2** If you detect 'other aroma', type in the description below.
**Sample __**

_____

_____

**Fig. 6.3** Ballot used for ultra-pasteurized milk shelf-life studies.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

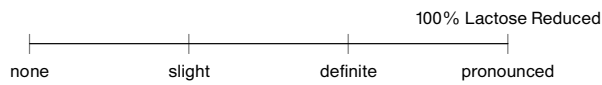
Downloaded From Vet-eBooks.com

**Question # 3 Please evaluate the following Flavor attributes for Sample __**

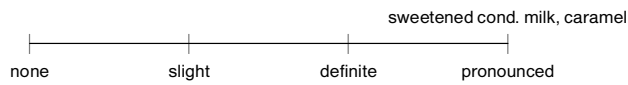
Cooked Flavor



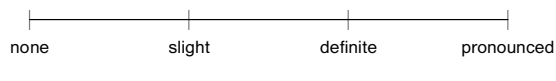

Sweet Flavor




Caramelized Flavor




Other Flavor



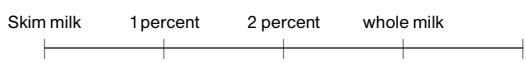

**Question # 4 If you detect 'other flavor', type in the description below. Sample __**

_____

_____

**Question # 5 Please evaluate the following Texture attributes for Sample __**

Viscosity




Chalky




Other Texture




**If you detect 'other texture', type in the description below.**

_____

_____

**Fig. 6.3** *continued*

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

**Question # 6 Please evaluate the following Aftertaste attributes for Sample __**

Bitter

none slight definite pronounced

Drying

Concorde grape

none slight definite pronounced

Lingering

5 sec.

none slight definite pronounced

Other Aftertaste

none slight definite pronounced

**If you detect 'other aftertaste', type in the description below.**

_____

_____

**Overall Quality Rating**

**Check scoring guide for off-flavors of Ultra-pasteurized milk posted in the booth.**

OVERALL QUALITY RATING

10 9 8 7 6 5 4 3 2 1 0

No Defects

**Fig. 6.3** *continued*

compounds (diacetyl, hexanol and $\delta$-decalactone) (Adhikari *et al.*, 2006). Prescott *et al.* (2005) used a method that combined a paired preference test with a threshold procedure to determine an average rejection point by consumers.

New understanding of sensory judgment has been obtained through comparison with physical and chemical data. Hotchkiss *et al.* (1999) studied the effect of dissolved $CO_2$ and barrier films on the shelf-life extension of milk. Once the sensory threshold was determined, the relationship between microbial

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

growth and package barrier properties to which $CO_2$ had been added at concentrations near the flavor threshold could be established. In a study by Hedegaard *et al.* (2006), descriptive sensory analysis was used to analyze the oxidative changes in milk. Sensory analysis and chemical analysis showed high correlation between the typical descriptors for oxidation, such as cardboard, metallic taste, and boiled milk and specific chemical markers for oxidation such as hexanal. Notably, primary oxidation products (i.e., lipid hydroperoxides), and even the tendency to form radicals as measured by electron spin resonance spectroscopy, showed high correlation to the sensory descriptors for oxidation.

An objective of many sensory evaluation studies is 'to provide useful chemical benchmarks of when off-flavors will be detected' (Santos *et al.*, 2003b, p. 2492). The following are two milk quality studies of this type. Santos *et al.* (2003a) used an ascending forced choice method (a series of triangle tests with ascending difference) to assess the sensory threshold for off-flavors in milk due to lipolysis and proteolysis resulting from the action of native milk enzymes. In 2% milk, lipolysis at a free fatty acid concentration of 0.25 meq/kg of milk was detected by more than one third of the panelists. For the detection of activity of native milk proteases in skim milk, the researchers found a detection sensory threshold at a 4.76% decline in casein as a percentage of true protein (CN/TP). Similarly, an off-flavor sensory detection threshold related to proteolysis was determined by Ma *et al.* (2000) to be about a 4% decline in CN/TP.

However, it must be remembered that detection does not necessarily mean rejection. Prescott *et al.* (2005) conducted two studies using a paired preference test with a method of constant stimuli threshold procedure to determine a consumer rejection threshold. Estimates of TCA thresholds in wine had been reported, but the level at which TCA became unacceptable had not. This study found that the detection threshold was 2.1 ppt, while the consumer rejection threshold was 3.1 ppt.

IP Address: 129.132.208.100

Often, several sensory techniques are applied in one study. Croissant *et al.* (2007) studied the impact of feed on the flavor of milk using the following methods: Spectrum descriptive analysis (to describe the flavor), a triangle difference test (to determine whether consumers could detect a difference between milk from conventional and pasture-based systems), a point hedonic scale (to determine consumer acceptance), and principal component analysis (PCA) (to determine how the treatments and individual sample collections differentiated from each other across sensory and instrumental measurements).

Descriptive sensory analyses are probably the most sophisticated tools in the arsenal of the sensory scientist. These techniques allow the sensory scientist to obtain complete sensory descriptions of products. They help identify underlying ingredient and process variables and/or determine which sensory attributes are important to acceptance. Usually, descriptive analyses yield objective descriptions of samples in terms of the perceived sensory attributes. One example of descriptive analysis is quantitative descriptive analysis (QDA). The principle of QDA is based on a panelist's ability to measure specific attributes of a product in a reproducible manner to yield a comprehensive quantitative

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

product description amenable to statistical analyses. In a QDA approach, panelists recruited from the general public work together in a focus group to identify key product attributes and appropriate intensity scales specific to a product. See Fig. 6.4 for examples of intensity line scales that are used in descriptive analysis. This group of panelists is then trained to reliably identify and score product attributes. As panelists generate the attribute terms, the resulting descriptions are meaningful to consumers.

Thus, analyses provide information amenable to modeling predictions of consumer acceptability. Physical reference standards determined by panel consensus are used to develop the proper descriptive language to reduce the amount of time required to train the panelists and to calibrate the panel in the use of the intensity scale. QDA results can be analyzed statistically and then represented graphically. The analytic technique of quantitative descriptive analysis has gained acceptance for sensory evaluation of various food and dairy products (Stone and Sidel, 2004b) and their shelf-life. Some examples of such studies include conventionally pasteurized milk (Phillips *et al.*, 1995; Quiñones *et al.*, 1998a,b) and microwave-treated milk vs. UHT milk (Clare *et al.*, 2005).

Chapman *et al.* (2001) used QDA to characterize the key attributes of nine UP milk products throughout their 60-day product shelf-life. The attribute terms were as follows: aroma attributes (cooked, caramelized, grainy/malty), flavor attributes (cooked, sweet, caramelized, bitter, metallic), texture attributes (viscosity, drying, chalky, lingering), and aftertaste attributes (drying, metallic, bitter). The 12 trained panelists evaluated the milk according to the method

IP Address: 129.132.208.100

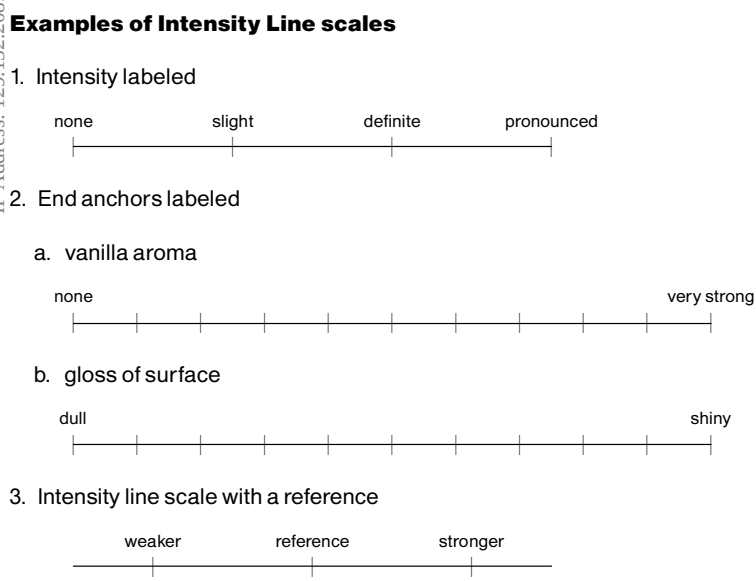



**Fig. 6.4** Examples of intensity line scales for measuring intensities of attributes.

© Woodhead Publishing Limited, 2010

Downloaded From Vet-x-Books.com

described in Chapman *et al.* (1998). The mean intensity of each attribute was graphically displayed in a 'spider plot' to provide a visual profile or 'fingerprint' of the important product attributes. Figure 6.2 shows a UP milk high in cooked aroma and cooked flavor, and medium in sweetness, caramelized flavor, drying, drying aftertaste, and lingering aftertaste. (Note that such a plot also shows that some taste attributes were *not* present, e.g., bitter flavor, chalky texture, bitter and metallic aftertaste.) Superimposing of spider plots can be used to compare products with each other or to compare attribute intensities of a single product tested at different points in time.

Lawless and Heymann (1998) indicated that data from sensory methods such as QDA, when combined with chemical or physical characteristics of a product detected, for example, by gas chromatographic olfactometry (GCO) or dynamic mechanical analysis (rheological measurements), respectively, can be used to define and optimize dairy product characteristics for different market segments. The combined sensory and instrumental data can then be analyzed with more advanced statistical protocols such as principal component analysis (PCA).

## 6.5 Application of advanced statistical methods

There are many new statistical methods available to the sensory scientist that can apply to dairy research. Some methods extend to marketing techniques such as conjoint analysis. Recently conjoint analysis has been used to simulate real-world consumer purchasing behavior. It also has been used to survey general knowledge and perception of health benefits of dairy products (Moskowitz *et al.*, 2005; Jones *et al.*, 2008). Conjoint analysis is also ideal for optimizing new product designs by identifying the most appealing sets of features. Conjoint analysis, sometimes referred to as trade-off analysis, is a multivariate technique that quantitatively measures the relative importance of different marketing variables, attributes, or product features related to a brand, product, or service. The distinguishing feature of this technique is that each variable's importance is determined implicitly or indirectly. That is, the respondent is not consciously aware of what is being measured.

The following papers illustrate some of the advanced methods of logistic regression, principal component analysis, and modeling. Logistic regression was used for the prediction of food shelf-life (Hough *et al.*, 2002, 2003; Gambaro *et al.*, 2004; Salvador and Fiszman, 2004). Chapman *et al.* (2006) used logistic regression for analyzing preference milk data. Because preference options can be reduced to a dichotomous response (preference/no preference), logistic regression is a useful tool for examining the impact of experimental manipulations on preferences (Hosmer and Lemeshow, 2000). Binomial (or binary) logistic regression is a form of regression that is used when the dependent variable is dichotomous and the independent variables are continuous or categorical. This procedure can be used to predict the probability of each response based on values of the independent variables, to rank the relative importance of independent

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

variables, to assess interaction effects, and to understand the impact of covariate control variables.

With descriptive sensory data, several dependent variables may be correlated with one another. To illustrate, following ANOVA, several sensory descriptors may appear to significantly discriminate among the samples, but multiple descriptors may be driven by the same underlying causes. Principal component analysis (PCA) is a multivariate technique that provides a method of extracting structure from a variance–covariance or correlation matrix. PCA identifies patterns of correlation among dependent variables and substitutes a new variable, called a factor, for the group of original attributes that were correlated. The analysis then identifies a second and a third group of attributes and derives a factor for each, based on the residual variance (that which is left after the variance accounted for by the previous factor has been removed). The attributes will have a correlation with the new dimensions, called a factor loading, and the products will have values on the new dimensions, called factor scores. The factor loadings are useful in interpreting the dimensions, and the factor scores show the relative positions among the products in a map (Lawless and Heymann, 1998). Thus, PCA transforms original dependent variables into new uncorrelated dimensions to simplify the data structure, eliminate descriptor redundancies, and indicate potential latent causal variables. That is, the factors may suggest underlying causes or processes that give rise to the resulting pattern of correlated changes in sensory attributes.

In a study with UP milk (Chapman *et al.*, 2001), PCA was applied to the mean attribute ratings listed to simplify interpretation of data from 15 attributes measured on nine products. PCA was applied with factor analysis (Lawless and Heymann, 1998). PCA was applied to the mean attribute ratings. The four principal components (PCs) generated from this analysis accounted for 94.4% of the total variance in the data set. The attributes selected each had a consistently high value, indicating that the attribute was often present, had low standard deviation, and was not highly correlated with another attribute. The analysis extracted the most significant variables with minimum loss of information. Varimax rotation was performed on these four PCs to bring them into closer alignment with the original variables. PC1 was entirely related to the following 'cooked' attributes: cooked aroma, grainy/malty aroma, and cooked flavor. PC2 had large negative loadings for dry and lingering. PC3 was largely negatively related to 'sweet' attributes: caramel and sweet. PC4 was almost entirely influenced by bitterness. Finally, the samples (products) were plotted into the data space described by the retained principal components. Factor scores were calculated for each sample to determine its location on the retained components. Samples farther apart on the principal component map are perceptually more different from samples found closer together (Coxon, 1982; Schiffman *et al.*, 1981).

After PCA analysis, a regression model can be used to estimate the overall product quality rating based on measurements of its attributes (Siebert, 1999). The overall quality ratings were modeled as a function of the four Varimax

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

rotated PCs for cooked, dry lingering, sweet and bitter scores for the UP products. Models were constructed using ordinary least squares, principal components regression, and partial least squares regression. The best fit equation for prediction of overall quality at day 60 was obtained (Chapman *et al.*, 2001):

$$\text{Overall quality rating} = 7.01 + 0.127\ \text{cooked} + 0.013\ \text{dry/lingering} + 0.154\ \text{sweet} - 0.424\ \text{bitter}$$

In general, as shown by the model, perception of bitter flavor had the most dramatic effect on overall quality perception.

Generation of quantitative descriptive sensory data can contribute to a well-defined competitive marketing strategy. Product positioning can assist a firm's target customers to understand and appreciate a specific product's characteristics in relation to those of its competitors' products. In this strategy, each brand within a set of competitive products is thought to occupy a certain position in a customer's 'perceptual space' (Urban *et al.*, 1987). In general, marketers have two broad objectives in mind when undertaking perceptual mapping. One objective is to determine where a target brand is positioned versus the competition. The other objective is to help identify determinant product attributes that influence customer choice within the product class (Kohli and Leuthesser, 1993). These determinant attributes must be important to customers and must also exhibit differences across brands. No matter how important a product attribute is, if brands are not perceived to differ in that attribute, then the attribute will not be influential in customers' decisions. Perceptual mapping can contribute to strategic product positioning for development and marketing of new products.

Sensory tests provide useful information about the human perception of product changes due to ingredients, processing packaging or shelf-life. Sensory results reduces decision risks. A well functioning sensory program will be useful to a company in meeting consumer expectations and ensuring a greater chance of marketing success. The utility of the information provided is directly proportional to the quality of the sensory measurement.

Statistics have always been an essential part of sensory evaluation. All quantitative sensory data should be analyzed statistically to be interpreted properly and to give actionable results. Even though statistics are necessary, they are only as good as the sensory data used.

## 6.6 Sources of further information and advice

- A 1974 survey conducted by Cornell University revealed a direct correlation between milk flavor and levels of milk consumption by school-age children (Boor, 2001). This clear link between milk quality and product consumption provided the initial impetus for the development of the Milk Quality Improvement Program (MQIP). Current objectives of the ongoing MQIP are

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

to monitor initial and keeping quality of commercially processed and packaged fluid milk products in New York State; to monitor raw milk quality; and to assist dairy plants in identifying and correcting handling and processing problems affecting dairy product quality. Sensory evaluation was used in monitoring HTST milk acceptability. Through the research and expertise of MQIP, nine of New York State's dairy plants have increased their shelf-life from 14 to 17 days. For more information contact Dr Kathryn J. Boor at Cornell University, Ithaca, NY.

- For practical procedures on conducting a taste panel, consult a good sensory evaluation text, like *Sensory Evaluation Techniques* (Meilgaard *et al.*, 2006). *Sensory Evaluation of Food: Principles and Practices* (Lawless and Heymann, 1998) is also helpful, and includes philosophical and psychological background. For statistical methods and procedures, consult *Sensory Evaluation of Food, Statistical Methods and Procedures* (O'Mahony, 1986). *Consumer Sensory Testing for Product Development* (Resurreccion, 1998) is excellent for consumer testing. *Sensory Evaluation of Dairy Products*, 2nd Edition (Clark *et al.*, 2009) is (1) an overview of the history, art, and science behind the sensory evaluation of dairy products; (2) a guide to assist in tracing the origins of identifiable sensory defects in dairy products with strategies for their correction; (3) a practical guide to the preparation of samples for sensory evaluation; and (4) a training tool for personnel in the evaluation of dairy products.
- ASTM International publishes standard specifications, tests, practices, guides, and definitions relating to materials, such as sensory methods for milk. See ASTM org or ASTM journal provides technical papers in print and online.
- The International Organization for Standardization (ISO), Geneva, Switzerland. ISO Standard 8586. 1. Sensory Analysis – General guidance for the selection, training, and monitoring of assessors.
- The Sensory User Group (Internet): visit sensory@yahoogroups.com.
- Institute of Food Technology (IFT).
- The Student Product Development Competition competes at the IFT annual meeting. They often develop new dairy products. IFT offers short courses. At the annual meeting are presenters from industry and academia (poster and oral presentations). The sensory evaluation division of IFT publishes a newsletter, called *Sensory Forum*, which tells about upcoming events and courses, employment opportunities, recent literature and more. Visit www.IFT.org.
- The Society of Sensory Professionals. Visit www.sensory.org/SSP/.
- Symposiums: *Pangborn Sensory Symposium* (www.pangborn2011.com), *Sensometrics Meeting* (www.sensometric.org).
- Journals in the field include *Journal of Sensory Studies* (published by Food & Nutrition Press, Inc.; view online resource from Blackwell Synergy), *Food Quality and Preference* (published by Elsevier Ltd, http://www.sciencedirect.com/science/journal/09503293), *Journal of Dairy Science* (published

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

by the American Dairy Science Association), *Journal of Food Science* (published by the Institute of Food Technology; view online resource from Blackwell Synergy), and *Dairy Foods* (published by BNP Media II, LLC, http://www.dairyfoods.com/).

- Some multivariate approaches to mapping product similarities are Drivers of Liking® and Landscape Sementation Analysis®, The Institute for Perception (ifpress@cs.com).
- Short courses are given by universities such as Cornell University (http://www.foodscience.cornell.edu/cals/foodsci/research/sensory/index.cfm) and UC Davis (www.extension.ucdavis.edu/sensory) and by companies such as International Resources for Insights and Solutions, The Institute for Perception (www.ifpress.com), Sensory Spectrum, Inc. (www/sensoryspectrum.com), Insights Now (Greg.Stucky@InsightsNow.com), and IRIS: International Resources for Insights and Solutions, LLC (amunoz@IRIS-consulting.net).
- Organizations that are involved in assessing, regulating, or promoting dairy products include:

Agricultural Marketing Service (AMS)
Ams.usda.gov

American Dairy Science Association
1111 B, Dunlap Avenue
Savoy, IL 61874
217-356-5146
Adsa.org

Center for Food Safety and Applied Nutrition
5100 Paint Branch Parkway
College Park, MD 20740-3835
Sfsan.fda.gov/list.html

Centers for Disease Control and Prevention (CDC)
1600 Clifton Road
Atlanta, GA 20222
1-800-311-3435
cdc.gov

Code of Federal Regulations (CFR)
Access.gpo.gov/cgi-bin/cfrassemble.cgi/title=200021
Collegiate Dairy Products Evalution Contest
Ams.usda/gpv/dairy/cdpec/coach_corner.htm

Collegiate Dairy Products Evaluation Contest
Ams.usda.gov/dairy/cdpec/coach_corner.htm

Department of Health and Human Services (HHS)
200 Independence Avenue, S.W.
Washington, DC 20201
hhs.gov

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

Future Farmers of America (FFA) represents a large diversity of over 300 careers in the food, fiber and natural resources industry. FFA (www.FFA.org) teaches sensory evaluation of milk.

International Dairy Foods Association (IDFA)
1250 W. Van Buren, Suite 900
Washington, DC 20005
202-737-4332
IDFA.org/index.cfm

National Dairy Council
Nationaldairycouncil.org/nationaldairycouncil/sitemap

Pasteurized Milk Ordinance (PMO)
Cfsan.fda.gov/~ear/pmo03toc.html

U.S. Food and Drug Administration (FDA)
5600 Fishers Lane
Rockville, MD 20857-0001
1-800-463-6332
Fda.gov

U.S. Public Health Service (PHS)
Phs.gov/

IP Address: 129.132.208.100

## 6.7 References and further reading

ACHANTA K, BOENEKE CA and ARYANA KJ (2007), 'Characteristics of reduced fat milks as influenced by the incorporation of folic acid', *J Dairy Sci*, 90, 90–98.

ADHIKARI K, HEIN K, ELMORE J, HEYMAN H and WILLMOTT A (2006), 'Flavor threshold as affected by interaction among three dairy-related flavor compounds', *J Sensory Studies*, 21, 626–643.

AMERINE MA, PANGBORN RM and ROESSLER EB (1965), *Principles of Sensory Evaluation*, New York, Academic Press.

ASTM (1992), Standard practice for determination of odor and taste threshold by a forced choice method of limits E-679-91. In: *Annual Book of Standards*. 15.07 Philadelphia, PA, American Society for Testing and Materials, pp. 35–39.

BARBANO D and LYNCH J (2006), 'Major advances in testing of dairy products: milk component and dairy product attribute testing', *J Dairy Sci*, 89, 1189–1194.

BODYFELT FW (1981), 'Dairy product score cards: are they consistent with the principles of sensory evaluation?', *J Dairy Sci*, 6, 2303–2308.

BODYFELT FW, TOBIAS J and TROUT GM (1988), *Sensory Evaluation of Dairy Products*, New York, Van Nostrand/AVI.

BOOR KJ (2001), 'ADSA Foundation Scholar Award Fluid Dairy Product Quality and Safety: Looking to the future', *J Dairy Sci*, 84, 1–11.

BOOR KJ and NAKIMBUGWE DN (1998), 'Quality and stability of 2% fat ultrapasteurized fluid milk products', *Dairy, Food Environ San*, 18, 78–82.

CHAPMAN KW and BOOR KJ (2001), 'Acceptance of 2% ultra-pasteurized milk by

© Woodhead Publishing Limited, 2010

Downloaded From Vet-eBooks.com

consumers, 6 to 11 years old', *J Dairy Sci*, 8, 951–954.

CHAPMAN KW, ROSENBERRY LC, BANDLER DK and BOOR KJ (1998), 'Light-oxidized flavor development and vitamin A degradation in chocolate milk', *J Food Sci*, 63, 930–934.

CHAPMAN KW, LAWLESS HT and BOOR KJ (2001), 'Quantitative descriptive analysis and principal component analysis for sensory characterization of ultrapasteurized milk', *J Dairy Sci*, 84, 12–20.

CHAPMAN KW, WHITED LJ and BOOR KJ (2002), 'Sensory threshold of light-oxidized flavor defects in milk', *J Food Sci*, 67, 2770–2773.

CHAPMAN KW, GRACE-MARTIN K and LAWLESS HT (2006), 'Expectations and stability of preference choice', *J Sensory Studies*, 21, 441–455.

CLAASSEN MR and LAWLESS HT (1992), 'A comparison of descriptive terminology systems for the sensory analysis of flavor defects in milk', *J. Food Sci*, 57, 596–621.

CLARE DA, BANG WS, CARTWRIGHT G, DRAKE MA, CORONEL P and SIMUNOVIC J (2005), 'Comparison of sensory, microbiological, and biochemical parameters of microwave versus indirect UHT fluid skim milk during storage', *J Dairy Sci*, 88, 4172–4182.

CLARK, S, COSTELLO, M, DRAKE M and BODYFELT FW (2009), *The Sensory Evaluation of Dairy Products*, New York, Springer Science.

COXON APM (1982), 'Three-way and further extensions of the basic model', in *The User's Guide to Multidimensional Scaling*, London, Heinemann Educational Books, pp. 186–241.

CROISSANT AE, WASHBURN SP, DEAN LL and DRAKE MA (2007), 'Chemical properties and consumer perception of fluid milk from conventional and pasture-based production systems', *J Dairy Sci*, 90, 4942–4953.

DEANE S (1797), *The New-England Farmer*, Worcester, MA, Isaiah Thomas, p. 78.

GAMBARO A, FISZMAN S, GIMENEZ A, VARELA P and SALVADOR A (2004), 'Consumer acceptability compared with sensory and instrumental measures of white pan bread: Sensory shelf-life estimation by survival analysis', *J Food Sci*, 69, 401–405.

GOFF HD and GRIFFITHS MW (2005), 'Major advances in fresh milk and milk products: fluid milk products and frozen desserts', *J Dairy Sci*, 89, 1163–1173.

HEDEGAARD RV, KRISTENSEN D, NIELSEN JH, FRØST MB, ØSTDAL H, HERMANSEN JE, KRÖGER-OHLSEN M and SKIBSTED LH (2006), 'Comparison of descriptive sensory analysis and chemical analysis for oxidative changes in milk', *J Dairy Sci*, 89, 495–504.

HOSMER DW and LEMESHOW S (2000), *Applied Logistic Regression*, 2nd edn, New York, John Wiley & Sons.

HOTCHKISS JH, CHEN JH and LAWLESS HT (1999), 'Combined effects of carbon dioxide addition and barrier films on microbial and sensory changes in pasteurized milk', *J Dairy Sci*, 82, 690–695.

HOUGH G, SANCHEZ RH, GARBARINI DE PABLO G, SANCHEZ RG, CALDERON VILLAPLANA S, GIMENEZ AM and GAMBARO A (2002), 'Consumer acceptability versus trained sensory panel scores of powdered milk shelf-life defects', *J Dairy Sci*, 85, 2075–2080.

HOUGH G, LANGOHR K, GOMEZ G and CURIA A (2003), 'Survival analysis applied to sensory shelf life of foods', *J Food Sci*, 68, 359–362.

JONES VS, DRAKE MA, HARDING R and KUHN-SHERLOCK B (2008), 'Consumer perception of soy and dairy products: a cross-cultural study', *J Sensory Studies*, 23:65–79.

KOHLI CS and LEUTHESSER L (1993), 'Product positioning: a comparison of perceptual mapping techniques', *J Prod Brand Mgt*, 2, 10–19.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

KROLL BJ (1990), 'Evaluating rating scales for sensory testing with children', *Food Technol*, 44, 78–86.

LAWLESS HT and CLAASSEN MR (1993), 'Validity of descriptive and defect-oriented terminology systems for sensory analysis of fluid milk', *J Food Sci*, 58, 108–112.

LAWLESS HT and HEYMANN H (1998), *Sensory Evaluation of Food: Principles and Practices*, New York, Chapman & Hall.

MA Y, RYAN C, BARABANO DM, GALTON DM, RUDAN MA and BOOR KJ (2000), 'Effects of somatic cell count on quality and shelf-life of pasteurized fluid milk', *J Dairy Sci*, 83, 264–274.

MATAK KE, SUMNER SS, DUNCAN SE, HOVINGH E, WOROBO RW, HACKNEY CR and PIERSON MD (2007), 'Effects of ultraviolet irradiation on chemical and sensory properties of goat milk', *J Dairy Sci*, 90, 3178–3186.

MEILGAARD M, CIVILLE GV and CARR BT (2006), *Sensory Evaluation Techniques*, 4th edn, Boca Raton, FL, CRC Press.

MOSKOWITZ HR, MUÑOZ AM and GACULA MC (2003), *Viewpoints and Controversies in Sensory Science and Consumer Product Testing*, Trumbull, CT, Food and Nutrition Press.

MOSKOWITZ HR, GERMAN JB and SAGUY IS (2005), 'Unveiling health attitudes and creating good-for-you foods: the genomics metaphor, consumer innovative web-based technologies', *Crit Rev Food Sci Nutr*, 45, 165–191.

O'MAHONY M (1986), *Sensory Evaluation of Food, Statistical Methods and Procedures*, New York, Marcel Dekker.

PERYAM DR (1958), 'Sensory difference tests', *J Food Tech*, 12, 231–236.

PHILLIPS LG and BARBANO DM (1997), 'The influence of fat substitutes based on protein and titanium dioxide on the sensory properties of lowfat milks', *J Dairy Sci*, 80, 2726–2731.

PHILLIPS LG, MCGIFF ML, BARBANO DM and LAWLESS HT (1995), 'The influence of nonfat dry milk on the sensory properties, viscosity, and color of lowfat milks', *J Dairy Sci*, 78, 1258–1266.

PRESCOTT J, NORRIS L, KUNST M and KIM K (2005), 'Estimating a "consumer rejection threshold" for cork taint in white wine', *Food Qual Pref*, 16, 345–349.

QUIÑONES HJ, BARBANO DM and PHILLIPS LG (1998a), 'Influence of protein standardization by ultrafiltration on the viscosity, color, and sensory properties of skim and 1% milk', *J Dairy Sci*, 80, 3142–3151.

QUIÑONES HJ, BARBANO DM and PHILLIPS LG (1998b), 'Influence of protein standardization by ultrafiltration on the viscosity, color, and sensory properties of 2 and 3.3% milks', *J Dairy Sci*, 81, 884–894.

RESURRECCION AVA (1998), *Consumer Sensory Testing for Product Development. Affective Testing with Children*, Gaithersburg, MD, Aspen Publishers.

RODRIGUEZ R (2007), 'Sensory and Nutritional Quality of Iron Fortified Milk', MS thesis, Cornell University.

ROLAND AM, PHILLIPS LG and BOOR KJ (1999), 'Effects of fat replacers on the sensory properties, color, melting, and hardness of ice cream', *J Dairy Sci*, 82, 2094–2100.

SALVADOR A and FISZMAN SM (2004), 'Textural and sensory characteristics of whole and skimmed flavored set-type yogurt during long storage', *J Dairy Sci*, 87, 4033–4041.

SANTOS MV, MA Y, CAPLAN Z and BARBANO DM (2003a), 'Sensory threshold of off-flavors caused by proteolysis and lipolysis in milk', *J Dairy Sci*, 86, 1601–1607.

SANTOS MV, MA Y and BARBANO DM (2003b), 'Effect of somatic cell count on proteolysis

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-eBooks.com

and lipolysis in pasteurized fluid milk during shelf-life storage', *J Dairy Sci*, 86, 2491–2503.

SCHIFFMAN SS, REYNOLDS ML and YOUNG FW (1981), *Introduction to Dimensional Scaling*, New York, Academic Press.

SCHUTZ HG and CARDELLO AV (2000), 'A labeled affective magnitude (LAM) scale for assessing food liking/disliking', *J Sensory Studies*, 16, 117–156.

SHIPE WF (1980), 'Analysis and control of milk flavors', in *The Analysis and Control of Less Desirable Flavors in Foods and Beverages*, G. Charalambous (ed.), Orlando, FL, Academic Press, pp. 201–239.

SIEBERT KJ (1999), 'Modeling the flavor threshold of organic acids in beer as a function of their molecular properties', *Food Qual Pref*, 10, 129–137.

STONE H and SIDEL JL (2004a), *Sensory Evaluation Practices*, 2nd edn, San Diego, Academic Press.

STONE H and SIDEL JL (2004b), 'Quantitative descriptive analysis: developments, applications, and the future', *Food Technol*, 52, 48–52.

TROUT GM, WHITE W, MACK MJ, DOWNS PA and FOUTS EL (1939), 'History and development of the Students' National Contest in the judging of dairy products', *J Dairy Sci*, 22, 375–387.

URBAN GL, HAUSER JR and DHOLAKIA N (1987), 'Mapping consumers' product perception', in *Essentials of New Product Management*, Englewood Cliffs, NJ, Prentice-Hall, pp. 103–120.

WHITED LJ, HAMMOND BH, CHAPMAN KW and BOOR KJ (2002), 'Vitamin A degradation and light-oxidized flavor defects in milk', *J Dairy Sci*, 85, 351–354.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

# 7

# Instrumental measurement of milk flavour and colour

**K. Cadwallader, University of Illinois, USA**

**Abstract:** Flavour and colour are among the main determinants of milk product quality. The understanding of how certain variables, such as production, processing and storage, impact the flavour and colour of fluid and dried milk products is of great interest to dairy scientists. For this reason instrumental methods have been developed for the measurement of the flavour and colour properties of milk products. Colour measurement is usually made using mature technologies based on colorimeters or spectrophotometers. On the other hand, the analysis of the flavour of milk and milk products presents a unique challenge and improved analytical methods are in constant development. This chapter presents a detailed discussion of modern instrumental methods used for the analysis of the flavour components and colour attributes of fluid and dried milk products.

**Key words:** flavour, odour, colour, milk, dried milk, dairy, gas chromatography, electronic nose, colorimeter.

IP Address: 129.132.208.100

## 7.1 Introduction

In the past two decades considerable progress has been made towards our understanding of the flavour chemistry/biochemistry of dairy products, but defining milk flavour continues to challenge researchers working in the sensory and flavour science fields. Fresh milk (raw, pasteurized and/or homogenized) of good quality has a subtle, yet distinctive clean and fresh dairy flavour. According to Badings (1991) there are three basic elements responsible for the sensory properties of milk, including: (1) pleasant mouth-feel due to presence of

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

macromolecules, such as colloidal proteins and fat globules, (2) sweet and salty taste due to lactose and milk salts, respectively, and (3) a weak and delicate aroma profile due to the proper balance of numerous volatile constituents found in low abundance. These elements, and to some extent the colour and appearance attributes, are the main determinants of the sensory quality of milk. Production, processing and storage practices can profoundly affect milk quality. For this reason the dairy industry has been actively engaged in the development of methods for the measurement of milk flavour and colour.

This chapter presents an overview of the instrumental methods used for the analysis of the flavour components and colour of fluid and dried milk products.

## 7.2 Flavour and colour of milk

Over the past five decades numerous researchers have demonstrated the involvement of carbonyls, esters, alcohols, short-chain free fatty acids, aromatics, sulphur-containing compounds and other miscellaneous compounds in the flavour of milk and milk products. Characteristic aroma components of fluid and dried milks that have been identified using gas chromatography–olfactometry methods are listed in Tables 7.1 and 7.2. The flavour components of milk are derived from two main sources: (1) feed constituents and rumen and animal metabolism during milk formation, and (2) reaction of milk protein, fat or lactose during processing and storage of milk (Forss, 1979; Badings, 1991). Before, during and after processing, the flavour of milk can be negatively affected by numerous chemical or biochemical reactions and processes (Forss, 1979). These include feed source, oxidative and hydrolytic rancidity, thermal degradation, packaging interactions and microbial contamination (Table 7.3). Several excellent reviews have been published on the volatile flavour components of milk and milk products (Forss, 1979; Forss and Sugisawa, 1981; Adda, 1986; Badings, 1991; Nursten 1997; Singh *et al.*, 2007; Cadwallader and Singh, 2009). In particular, the review by Cadwallader and Singh (2009) presents a detailed discussion of the occurrence and formation of flavour and off-flavour compounds in milk and dairy products.

In contrast to flavour, very little attention has been paid to the appearance characteristics, and, in particular, the colour of milk and dried milk. As pointed out by Burton (1956) the reason for this might be because the colour of milk is generally taken for granted and doesn't become a quality issue until it is altered from its normal or expected state. Fluid milk varies in colour from opaque white to yellowish-white or even to a blueish tinted white (Doan, 1924). The observed colour of milk is attributed to the light reflectance properties of the milk components such as fat globules, colloidal substances (e.g., proteins), $\beta$-carotene and riboflavin (Solah *et al.*, 2007). From a quality standpoint it has mainly been the discolouration of milk caused by Maillard (nonenzymatic) browning that has concerned the dairy industry. Colour changes in pasteurized fluid milk are generally minimal and are usually not of much concern. On the other hand,

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

**Table 7.1** Potent odourants identified by gas chromatography–olfactometry in raw, pasteurized and UHT fluid milks (compounds listed in decreasing order of relative odour potency)

| *Raw milk (whole)*[a,b] | *Pasteurized milk (whole)*[b] | *Pasteurized milk (whole/skim blend)*[e] | *UHT milk (glass bottles)*[f] |
|---|---|---|---|
| ethyl hexanoate | dimethyl sulphone | 1-octen-3-one | δ-decalactone |
| ethyl butanoate | hexanal | dimethyl sulphide | vanillin |
| dimethyl sulphone | nonanal | hexanoic acid | 6-(*Z*)-dodecenyl-γ-lactone |
| nonanal | 1-octen-3-ol | 2-methylthiophene | γ-dodecalactone |
| 1-octen-3-one | indole | acetic acid | *trans*-4,5-epoxy-(*E*)-2-decenal |
| indole | benzothiazole | phenylacetaldehyde | δ-octalactone |
| heptanal | δ-decalactone | 1-nonen-3-one | 2-acetyl-2-thiazoline |
| ethyl octanoate | 2-tridecanone | dimethyl trisulphide | 3-(methylthio)propanal (methional) |
| octanal | | 2,3-butanedione | 2-acetyl-1-pyrroline |
| | *Pasteurized milk (skim, 2% and whole)*[d] | 3-methyl-2-butenal | |
| *Raw milk (whole)*[c] | 2-acetyl-1-pyrroline | benzothiazole | *UHT milk (PE bottles)*[f] |
| 3-(methylthio)propanal (methional) | dimethylsulphide | 2-methoxyphenol (guaiacol) | δ-decalactone |
| δ-decalactone | methylpropanal | | γ-dodecalactone |
| 2-acetyl-1-pyrroline | 3-methylbutanal | *UHT milk*[b] | 3-methoxy-4-hydroxybenzaldehyde (vanillin) |
| 2-acetyl-2-thiazoline | 1-octen-3-one | 2-heptanone | (*Z*)-4-heptenal |
| 6-(*Z*)-dodecenyl-ã-lactone | octanal | 2-nonanone | 3-(methylthio)propanal (methional) |
| 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (furaneol) | 1-hexen-3-one | 2-undecanone | hexanoic acid |
| 2-isopropyl-3-methoxypyrazine | methanethiol | δ-decalactone | δ-octalactone |
| 3-methylindole (skatole) | hexanal | 2-tridecanone | *trans*-4,5-epoxy-(*E*)-2-decenal |
| (*E,Z*)-2,6-nonadienal | 3-methylindole (skatole) | dimethyl sulphone | γ-nonalactone |
| δ-octalactone | 1-nonen-3-one | benzothiazole | δ-nonalactone |
| decanoic acid | | hexanal | |
| (*E*)-2-nonenal | | indole | |
| (*E,E*)-2,4-nonadienal | | | |
| γ-decalactone | | | |
| 1-octen-3-one | | | |

[a] Moio *et al.* (1993). [b] Moio *et al.* (1994). [c] Colahan-Sederstrom and Peterson (2005). [d] Cadwallader and Howard (1998). [e] Ott *et al.* (1997).
[f] UHT milks stored in either glass or polyethylene (PE) bottles (Czerny and Schieberle, 2007).

© Woodhead Publishing Limited, 2010
IP Address: 129.132.208.100

Downloaded From Vet-eBooks.com

© Woodhead Publishing Limited, 2010
IP Address: 129.132.208.100

**Table 7.2** Potent odourants identified by gas chromatography–olfactometry in nonfat and wholefat dried milks (data for products having normal flavour quality; compounds listed in decreasing order of relative odour potency)

| *Nonfat dry milk (low heat)*[a] | *Nonfat dry milk (medium heat)*[a] | *Nonfat dry milk (high heat)*[a] |
|---|---|---|
| 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (furaneol) | δ-decalactone | 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (furaneol) |
| butanoic acid | 4-hydroxy-2,5-dimethyl-3(*2H*)-furanone (Furaneol) | butanoic acid |
| 3-(methylthio)propanal (methional) | butanoic acid | 3-(methylthio)propanal (methional) |
| *o*-aminoacetophenone | 3-(methylthio)propanal (methional) | *o*-aminoacetophenone |
| δ-decalactone | *o*-aminoacetophenone | *trans*-4,5-Epoxy-(*E*)-2-decenal |
| octanoic acid | *trans*-4,5-epoxy-(*E*)-2-decenal | δ-decalactone |
| pentanoic acid | 3-methoxy-4-hydroxybenzaldehyde (vanillin) | pentanoic acid |
| 3-methoxy-4-hydroxybenzaldehyde (vanillin) | 2-acetyl-1-pyrroline | 3-hydroxy-4,5-dimethyl-2(*5H*)-furanone (sotolon) |
| 2-acetyl-1-pyrroline | 2-acetyl-2-thiazoline | 3-methoxy-4-hydroxybenzaldehyde (vanillin) |
| hexanoic acid | 3-hydroxy-4,5-hydroxy-2(*5H*)-furanone (sotolon) | phenylacetic acid |
| nonanal | hexanoic acid | nonanal |
| 2-acetyl-2-thiazoline | phenylacetic acid | 1-octen-3-one |
| γ-dodecalactone | γ-dodecalactone | 2-acetyl-1-pyrroline |
| (*E*)-2-nonenal | (*E*)-2-undecenal | hexanoic acid |
| (*E*)-2-undecenal | (*E,E*)-2,4-decadienal | octanoic acid |
| *trans*-4,5-epoxy-(*E*)-2-decenal | 3-phenylpropanoic acid | γ-dodecalactone |
| 3-hydroxy-4,5-hydroxy-2(*5H*)-furanone (sotolon) | | (E)-2-nonenal |
| phenyl acetic acid | | 3-phenylpropanoic acid |
| 1-octen-3-one | | 3-hydroxy-2-methyl-*4H*-pyran-4-one (maltol) |
| (*E,E*)-2,4-decadienal | | |
| 3-phenylpropanoic acid | | |
| 3-hydroxy-2-methyl-*4H*-pyran-4-one (maltol) | | |
| 2-methylpropanoic acid | | |

Downloaded From Vet-eBooks.com

© Woodhead Publishing Limited, 2010
IP Address: 129.132.208.100

| *Nonfat dry milk*[b] | phenylacetaldehyde | δ-dodecalactone |
|---|---|---|
| 2-acetyl-1-pyrroline | acetic acid | 3-hydroxy-2-methyl-*4H*-pyran-4-one (maltol) |
| hexanoic acid | nonanal | 3-hydroxy-4,5-dimethyl-2(*5H*)-furanone |
| 4-hydroxy-2,5-dimethyl–3(*2H*)-furanone | (*E,Z*)-2,6-nonadienal | (sotolon) |
| (Furaneol) | propanoic acid | δ-decalactone |
| δ-octalactone | pentanoic acid | 2/3-methylbutanoic acid |
| *o*-aminoacetophonene | 2-acetylthiazole | 2-methylthiophene |
| 3-(methylthio)propanal | 2-acetyl-2-thiazoline | 4-hydroxy-2-ethyl-5-methyl-3(*2H*)-furanone |
| γ-dodecalactone | 3-methylindole | (homofuraneol) |
| γ-octalactone | | (*E*)-2-decenal |
| 4-hydroxy-2-ethyl-5-methyl-3(*2H*)-furanone | | 2-acetylthiazole |
| (homofuraneol) | *Wholefat dry milk*[b] | (*E*)-2-nonenal |
| 1-octen-3-one | hexanoic acid | 2-acetyl-2-thiazoline |
| 3-hydroxy-4,5-dimethyl-2(*5H*)-furanone | 3-methylindole (skatole) | propanoic acid |
| (sotolon) | 2-acetyl-1-pyrroline | pentanoic acid |
| 6-(Z)-dodecenyl-γ-lactone | *o*-aminoacetophonene | 1-octen-3-one |
| butanoic acid | γ-octalactone | 2-methoxyphenol |
| octanoic acid | γ-dodecalactone | 2,3-butanedione |
| 2/3-methylbutanoic acid | butanoic acid | 3-(methylthio)propanal (methional) |
| phenylacetaldehyde | δ-octalactone | nonanal |
| octanoic acid | acetic acid | (*E,E*)-2,4-nonadienal |
| 2/3-methylbutanoic acid | octanoic acid | phenylacetaldehyde |

[a] Karagül-Yüceer *et al.* (2001, 2003).
[b] Carunchia Whetstine and Drake (2007).

Downloaded From Vet-eBooks.com

© Woodhead Publishing Limited, 2010
IP Address: 129.132.208.100

**Table 7.3** Off-flavours and flavour defects in fluid and dried milks

| Flavour defect | Chemicals | Origin | References |
|---|---|---|---|
| Feed | Dimethyl sulphide, acetone, butanone, isopropanol, ethanol, propanal | Metabolites of feed and silage | Badings (1991) |
| | (*E*)-2- and (*Z*)-2-nonenal | Fresh cut alfalfa | Marsili (2003) |
| | Dimethyl sulphide, 2-butanone, hexanal | Grass silage | Mounchili *et al.* (2005) |
| | Terpenes | Grass (pasture) | Schlichtherle-Cerny *et al.* (2007) |
| Fishy | Trimethylamine | Microbial | Cornfield (1955); Humphriss (1953); Lunden *et al.* (2002); Ampuero *et al.* (2002) |
| Fruity | Ethyl esters (e.g., ethyl butanoate, ethyl hexanoate) | Microbial, enzymatic | Wellnitz-Ruen *et al.* (1982); Whitfield *et al.* (2000); Marsili (2003) |
| Heat abuse, cooked | Maltol, furans, pyrazines and other Maillard reaction products | Thermally induced | Badings (1991) |
| | $H_2S$, methanethiol, dimethylsulphide and other sulphides, 2-alkanones, methylpropanal, 3-methylbutanal, *n*-aldehydes | Thermally induced | Contarini *et al.* (1997); Boelrijk and de Jong (2003); Marsili and Miller (1998); Vazquez-Landaverde *et al.* (2005, 2006) |
| Light-abuse | Dimethyl sulphide, dimethyl disulphide, *n*-aldehydes, 2-alkanones, vinyl ketones | Light-induced oxidation | Mehta and Bassette (1979); Jeng *et al.* (1988); Cadwallader and Howard (1998); Jung *et al.* (1998), Kim and Morr (1996); Marsili and Miller (1998); van Aardt *et al.* (2005) |

Downloaded From Vet-eBooks.com

| | | | |
|---|---|---|---|
| Oxidized | *n*-Aldehydes, 2/3-alkenals, 2,4-alkadienals, 2,6-nonadienal, 2,4,7-decatrienal, ketones, vinyl ketones | Lipid oxidation (e.g., copper-induced) | Forss *et al.* (1955a,b); Day *et al.* (1963), Hammond and Seals (1972); Badings (1991); Barrefors *et al.* (1995); Granelli *et al.* (1998); Marsili and Miller (1998) |
| | (*E*)-6-Nonenal | Ozonolysis | Parks *et al.* (1969) |
| Phenolic | *p*-Cresol | Enzymatic, enzymatic | Badings and Neeter (1980) |
| Rancid | Volatile short-chain fatty acids (C4–C10) | Microbial, enzymatic, sanitizer | Azzara and Campbell (1992); Marsili (2000); Whitfield *et al.* (2000) |
| Stale, storage | 2-Alkanones, *o*-aminoacetophenone, Strecker aldehydes (e.g., 3-methylbutanal, methional), *n*-aldehydes, 2-alkenals, 2,4-alkadienals, vinyl ketones | Storage (lipid-oxidation, Maillard reaction) | Arnold *et al.* (1966); Parks *et al.* (1964); Anderson and Lingnert (1998); Preininger and Ullrich (2001); Valero *et al.* (2001); Karagül-Yüceer *et al.* (2002); Perkins *et al.* (2005); Carunchia Whetstine and Drake (2007) |
| Taints, chemical | Propylacetate | Solvent residue from packaging | Marsili (2003) |
| Taints, weed | Indole, skatole, mercaptans, sulphides, nitriles, thiocyanates | Metabolites of weeds | Badings (1991) |
| | Skatole, benzylthiol, benzyl methyl sulphide | Metabolites of weeds | Park (1969); Park *et al.* (1969) |

© Woodhead Publishing Limited, 2010
IP Address: 129.132.208.100

Downloaded From Vet-eBooks.com

browning reactions in UHT, condensed and dried milks can be considerable and may affect product quality (Patton, 1955; Popov-Raljić *et al.*, 2008). Several factors are known to promote browning reactions in milk products, including use of excessive processing temperatures and adverse storage conditions. Heating of fluid milk has two main effects on its colour: at moderate temperatures (65ºC) or immediately after a UHT process an initial 'whitening' may occur, while at high temperatures (>90ºC) browning readily occurs due to the Maillard reaction (Burton, 1955, 1956; Kessler and Fink, 1986; Rhim *et al.*, 1988; Singh and Creamer, 1992; Browning *et al.*, 2001; Popov-Raljić *et al.*, 2008). The increased rate of the Maillard reaction in concentrated and dried milks is due to the lower water activities and increased concentrations of reactant molecules in these products (van Boekel, 1998).

## 7.3 Flavour measurement

The availability of powerful chromatographic separation techniques, such as high-resolution capillary gas chromatography–mass spectrometry (GC-MS) and GC–olfactometry (GCO), has allowed for the detailed identification and characterization of critical flavour components of milk. In addition, the development of various types of electronic noses has provided the dairy industry with a rapid means to assess milk flavour quality. Reviews by Mairaca and Bosset (1997), Parliment and McGorrin (2000), McGorrin (2007), Singh *et al.* (2003), Cadwallader (2007) and Drake *et al.* (2007) describe some of the analytical techniques used in the evaluation of key aroma compounds in milk and dairy products. The book *Sensory-directed Flavor Analysis* edited by Marsili (2007a) is highly recommended to those readers interested in greater detail about the general analytical techniques used in flavour research. The review by Ampuero and Bosset (2003) provides important insights into the application of electronic nose technology for the analysis of dairy products.

The initial and often the most critical step in aroma analysis is the isolation of the volatile compounds from the nonvolatile matrix components. Following isolation, instrumental methods of analysis, such as GC-MS, are employed to separate, identify and quantify the various volatile components of the isolate. In some cases, combined sensory–instrumental methods, such as GC–olfactometry (GCO), can be applied to indicate important contributors to the characteristic aroma of the product. This section focuses on modern procedures used for the isolation and analysis of volatile flavour compounds of fluid and dried milk products.

### 7.3.1 Flavour compound isolation/extraction

The analysis of the volatile components of milk is a difficult process due to the presence of only minute amounts of volatile solutes in a highly complex nonvolatile matrix. Furthermore, milk volatile compounds comprise many

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

different chemical classes, such as acids, ketones, aldehydes, alcohols, etc., and are subject to further chemical breakdown due to oxidation and thermal decomposition. For this reason the volatile isolation procedure is usually the most critical step in the analysis. The isolation of volatile constituents may be accomplished by considering the differences in chemical properties between the volatile compounds and nonvolatile milk matrix components. Generally, this means taking advantage of the higher volatility and/or the relative nonpolar nature of the volatile components. Methods that depend on the volatile nature of the aroma components include headspace and distillation techniques. On the other hand, solvent extraction and adsorption methods rely on the relative nonpolar nature of the volatile compounds to isolate them from the matrix. A good approach is to use a method that takes advantage of both volatility and polarity differences in the isolation step, such as combined headspace–adsorption and extraction–distillation methods.

There is no single 'perfect' volatile isolation procedure and all volatile isolation techniques will impart some degree of sampling bias (Etiévant, 1996; Reineccius, 2006). For greater accuracy the method chosen should maintain sample integrity, minimize loss of labile (sensitive) aroma compounds, and isolate all volatile compounds equally. To help assure accuracy, it is common practice to use two or more complementary isolation methods that are based on different separation criteria. In this way, the sampling bias of each method can be accounted for in the final results. Methods most commonly employed for the isolation of volatile constituents of milk products are discussed below.

IP Address: 129.132.208.100

*Headspace methods*

All aroma compounds possess some degree of volatility. Headspace isolation techniques take advantage of this property by monitoring the gaseous headspace above a liquid or solid material in a sealed container. Headspace methodologies, including static headspace, dynamic headspace and purge-and-trap, have been reviewed (Hinshaw, 1990; Cole and Woolfenden, 1992; Wampler, 2002). Some unique advantages of headspace methods are that they provide some indication of the composition of the volatiles above the food (headspace aroma composition), they are nondestructive (mild conditions), and minimal sample preparation is required.

Static headspace analysis

In principle, static headspace analysis (SHA) is the simplest among the headspace analysis techniques. In SHA the food product (liquid or solid) is contained in a closed vessel (typically a vial is used), and the volatile components are allowed to come into equilibrium between the sample matrix and the surrounding headspace. For analysis, an aliquot of the headspace is then withdrawn and injected into a GC. SHA is influenced by numerous parameters, such as temperature, sample and container size, ratio of sample to headspace volume, nature of the sample matrix, addition of matrix modifiers (salting out) and whether or not the sample is stirred or agitated during equilibration. Advantages

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

of SHA include quick and simple sample preparation, elimination of reagents (no solvent peak during GC analysis) and low risk of artifacts. The main disadvantage of SHA is that it is limited to products that contain appreciable levels of highly volatile 'headspace' components. SHA may be a good choice for targeted analyses of some highly volatile components of milk, such as acetaldehyde, hydrogen sulphide, diacetyl, methanethiol, dimethylsulphide and low molecular weight Strecker aldehydes (e.g. methylpropanal, 2-methylbutanal and 3-methylbutanal). SHA has been successfully used for the analysis of highly volatile sulphur compounds in heated milk (Christensen and Reineccius, 1992) and for general volatile profiling of fluid milk (Toso *et al.*, 2002).

### Dynamic headspace analysis

The sensitivity of headspace analysis can be greatly improved by use of an intermediate trapping or adsorption step to enrich the volatile constituents prior to GC analysis. This technique is commonly referred to as dynamic headspace analysis (DHA) or purge-and-trap analysis and is useful for the analysis of trace-level components of high and intermediate volatility. In DHA an inert gas (nitrogen or helium) is swept over or through a thermostatted sample (contained in a closed vessel) for a period of time sufficiently long enough to 'extract' the volatile constituents. During this process the volatiles are enriched by an intermediate trapping step facilitated by use of adsorbent materials (porous polymers or charcoal) or by cryogenic focusing. Adsorbent trapping is most commonly used since it avoids the trapping of water vapour, which can adversely affect the cryogenic injector or GC column performance. Immediately after collection, trapped volatiles are transferred, most commonly by thermal desorption, to the GC for subsequent analysis. In order to improve GC performance, it is common practice to cryofocus the thermally desorbed votatiles in the GC inlet prior to analysis. Applications of adsorbent trapping–thermal desorption techniques are discussed in greater detail elsewhere (Hartman *et al.*, 1993; Butrym, 1999; Wampler, 2002).

DHA has similar advantages to SHA in that there is only minimal sample preparation required, no reagents are used, which means there is no solvent peak during GC analysis, and there is a low risk of artifact formation due to sample decomposition. A major limitation is that DHA is not an efficient method for analysis of semi-volatile compounds. Due to its ease of use, high sample throughput and relatively low cost per unit analysis, DHA has been the most extensively used method for the analysis of volatile components of fluid and dried milk products (Wellnitz-Ruen *et al.*, 1982; Park and Goins, 1992; Vallejo-Cordoba and Nakai, 1993; Imhof and Bosset, 1994; Barrefors *et al.*, 1995; Kim and Morr, 1996; Señorans *et al.*, 1996; Anderson and Lingnert, 1998; Cadwallader and Howard, 1998; Granelli *et al.*, 1998; Rysstad *et al.*, 1998; Valero *et al.*, 1999, 2001; Contarini and Povolo, 2002; Toso *et al.*, 2002; Fernandez *et al.*, 2003; Solano-Lopez *et al.*, 2005; Schlichtherle-Cerny *et al.*, 2007).

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

Static headspace–solid phase microextraction

In the past decade static headspace–solid phase micro extraction (HS-SPME) has emerged as one of the most commonly used methods for the isolation of volatile compounds from foods. HS-SPME is a rapid, solventless technique and is based on the partitioning of the volatile components between the sample headspace and a polymer-coated fibre. For analysis, the adsorbed volatiles are thermally desorbed in the heated inlet of the GC. The application of HS-SPME in the analysis of volatiles in foods has been reviewed and critically evaluated (Roberts *et al.*, 2000; Harmon, 2002; Marsili, 2002b; Werkhoff *et al.*, 2002; Vas and Vékey 2004).

Several adsorbent phases and film thicknesses are available for SPME. All the parameters of SHA should be considered in HS-SPME, as well as the nature of the fibre coating and fibre exposure (extraction) time. It is very important that the chosen sampling temperature does not result in any sample decomposition. Roberts *et al.* (2000) recommended the use of short extraction times (1–5 min) for highly volatile compounds and longer extraction times (5–30 min) for semi-volatile compounds. Transfer of the volatiles from the fibre to the GC is generally accomplished using hot, splitless injection, which may result in loss of some thermally labile volatile compounds.

Contarini and Povolo (2002) reported that DHA and HS-SPME provided similar precision, but differed in their recovery factors, for the analysis of volatiles in fluid milks subjected to different heat treatments. HS-SPME has also been applied for the study of milk with off-flavours (Whitfield *et al.*, 2000; Mounchili *et al.*, 2005; Hedegaard *et al.*, 2006) and thermally derived off-flavours (Vazquez-Landaverde *et al.*, 2005), and to measure volatile profiles of high pressure processed milks (Vazquez-Landaverde *et al.*, 2006, 2007).

IP Address: 129.132.208.100

### 7.3.2 Solvent extraction–distillation methods

The combination of distillation with solvent extraction has been extensively used for the isolation of volatile compounds from foods (Chaintreau, 2001; Parliment, 2002). The traditional method of simultaneous distillation–solvent extraction (SDE) has, in recent years, been replaced by milder methods, e.g. direct solvent extraction–high vacuum distillation, that avoid the formation of thermally generated artifacts due to sample (thermal) decomposition and that minimize the loss of thermally labile volatile components.

*Direct solvent extraction*

Most volatile organic compounds in foods are considerably less polar than the bulk, mostly aqueous food matrix and, therefore, can be readily isolated by direct solvent extraction (DSE). The main concern with this approach for the analysis of milk volatiles is that the resulting solvent extract will contain appreciable amounts of nonpolar and nonvolatile lipids and minor amounts of other nonvolatile material. Therefore, these extracts should not be injected directly into a GC without first taking some precautionary steps. One such

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

approach has been to perform an additional cleanup step, such as high vacuum distillation, to isolate the volatile components of the extract from the nonvolatile material prior to GC analysis.

Direct solvent extraction is an effective means of isolating a broad range of volatile constituents from milk. It is especially suitable for extracting semi-volatile constituents (e.g. lactones, free fatty acids, phenolics, etc.) that cannot be effectively analysed using headspace methods. Repeated extractions are generally necessary to minimize some of the bias caused by the inherent selectivity of the solvent chosen for the extraction. A good general solvent is diethyl ether for several reasons: it has good selectivity toward most aroma compounds, it has a relatively low density, which enables ease of recovery, and it has a low boiling point so that it can be evaporated without significant losses of the extracted volatile substances. An important consideration in solvent extraction is solvent purity. This includes water if it is used in the analysis or in the preparation of other reagents. It is prudent to run a blank to account for any impurities or artifacts introduced by the solvent or other reagents used in the analysis.

Researchers have employed direct solvent extraction for recovery of milk volatiles (Cadwallader and Howard, 1998; Polineni and Peterson, 2005). Once a solvent extract is prepared, it is usually subjected to a high vacuum distillation cleanup step, separated into neutral/basic and acidic fractions and then concentrated by removing the bulk of the extraction solvent prior to GC analysis. These steps are discussed below.

IP Address: 129.132.208.100

*High vacuum distillation*

High vacuum distillation is the method of choice for 'cleaning up' volatile extracts prepared by direct solvent extraction. This step is especially important if the aroma extract is to be analysed by cool, on-column GC or other injection technique where the nonvolatile material will either interfere with the injection or lead to formation of thermally generated artifacts if a heated GC inlet is used. A careful and highly efficient solvent assisted flavour evaporation (SAFE) distillation system was developed for high vacuum distillation of either liquid products or solvent extracts (Engel *et al.*, 1999). SAFE has been used, often in combination with DSE, for the preparation of aroma extracts from milk products (Carunchia Whetstine *et al.*, 2005, 2006, 2007; Colahan-Sederstrom and Peterson, 2005). SAFE has been shown to be effective for the extraction of most volatiles from solvent extracts of cheese, including difficult polar and semi-volatile constituents (Werkhoff *et al.*, 2002).

*Class fractionation and concentration of extracts*

Aroma extracts prepared by direct solvent extraction–high vacuum (SAFE) distillation often contain hundreds of volatile constituents of varying polarities and containing different functional groups. Various methods, such as adsorption column chromatography and preparative GC, can be used to simplify the analysis by fractionation of the extract prior to GC analysis. One of the simplest

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

and most common methods is the use of acid/base chemistry to fractionate the extract into its acidic, basic and neutral components. The advantage of performing this fractionation step is that the GC chromatograms are less crowded with peaks. Furthermore, one does not need to compromise as much on GC column selection, since the optimum column may be used for the different fractions. For milk products, it is generally not beneficial to fractionate the basic compounds from the neutral ones, since milk contains only a low abundance of basic volatile compounds (e.g. 2-acetyl-1-pyrroline, *o*-aminoacetophenone, indole and skatole).

The general approach for fractionation of aroma extracts has been to back-extract the acidic components from the solvent extract using aqueous bicarbonate or other suitable aqueous base (Carunchia Whetstine *et al.*, 2005). The neutral/basic components are retained in the organic solvent. The aqueous base is then acidified and then subjected to solvent extraction to yield the acidic fraction (contained in the organic solvent).

Prior to GC analysis, it is usually necessary to enrich the concentration of the volatile analytes in the aroma extract. This is most often accomplished by careful distillation (e.g., use of Kuderna–Danish concentration apparatus) or by slow evaporation of the solvent under a gentle stream of inert gas (e.g. nitrogen). A major drawback to this concentration step is that some volatile constituents are lost (evaporate) along with the extraction solvent. It is for this reason that direct solvent extraction is generally not suitable for the analysis of highly volatile components.

IP Address: 129.132.208.100

### 7.3.3 Other isolation/extraction methods

In addition to the methods described above, there are some new and emerging methods that have not yet been widely applied to the study of milk flavour. These include stir bar sorptive extraction (SBSE) (Baltussen *et al.*, 1999), solid-phase dynamic extraction (SPDE) (Bicchi *et al.*, 2004; Christ *et al.*, 2007) and single droplet microextraction (SDME) (Wood *et al.*, 2004). The main attraction of these methods is that they are suitable for use with multifunctional GC autosamplers.

### 7.3.4 Instrumental methods of analysis

*Gas chromatography–mass spectrometry*

Tandem gas chromatography–mass spectrometry (GC-MS) is the method of choice for the analysis of volatile food components. The pre-eminence of GC-MS is due to the fact that high resolution GC provides the highest overall efficiency and performance of all separation methods and is readily operated in tandem with MS.

Present day GC involves the use of high resolution open tubular columns with bonded phases that are capable of separating hundreds of volatile constituents in a single run. GC is a mature methodology and the theory and general application

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

will not be discussed here. Two critical parameters that can profoundly influence the results of GC, injection technique and analytical column stationary phase, are discussed below.

The injection inlet is used to introduce the volatile compounds, typically 1 to 3 $\mu$L of an aroma extract or 1 to 25 mL of headspace vapour, into the GC column. Programmable temperature vaporizer (PTV) and on-column injectors represent the best options among the many available GC injectors. The PTV inlet has particular appeal since it allows for cryogenic focusing and ramped heated (programmable) injections in either split or splitless modes among other possible configurations. Cool on-column injection has been the method of choice for analysis of aroma extracts prepared by direct solvent extraction–high vacuum distillation since it avoids thermal degradation and all of the components of the extract are completely introduced into the GC column (i.e. there is no injector discrimination).

In general, only two types of GC phases are required for the identification and quantitation of the volatile constituents of milk. Polarity of the stationary phase is the most important parameter and should be matched as closely as possible with the polarity of the analytes. For identification purposes it is best to determine retention indices for each analyte on two columns of differing polarity. Polyethylene glycol phases, such as a Carbowax (e.g. DBWAX) or free fatty acid phase (e.g. FFAP), are suitable for the analysis of most polar compounds. A 95% dimethyl–5% diphenylpolysiloxane phase (e.g. DB5) is commonly used for the analysis of nonpolar compounds. Some compounds are incompatible with certain phases, e.g. short-chain free fatty acids do not separate well on nonpolar phases. In such cases it may be necessary to use an intermediate polarity phase, e.g., 86% dimethyl–14% cyanopropylphenylpolysiloxane (DB-1701). Blank (2002) presented a detailed discussion of the importance of GC stationary phase in aroma research.

Many detectors are available for GC. The flame ionization detector (FID) is most commonly used for routine GC; however, for the analysis of highly complex volatile mixtures a mass spectrometric (MS) detector is preferred since it provides both qualitative and quantitative information. The main advantage of GC-MS is that it allows for mass spectral library matching for identification of unknown chromatographic peaks. The total ion chromatogram generated by full scan GC-MS can also be used for routine peak quantitation. For analysis of trace constituents, selected ion monitoring (SIM) mass spectrometry and 'mass chromatography' are often used. In SIM only selected ions representative of a specific compound or class of compounds are recorded during GC-MS analysis. The technique provides high sensitivity for analysis of known constituents but does not provide any useful information for the identification of unknown compounds, since full mass spectra are not recorded. Mass chromatography can be considered as retrospective SIM. With this method complete mass spectra are recorded throughout the GC-MS run rather than just selected ions. The data analysis software can then be used to re-plot only specific ions from full spectra data, with the aim of resolving co-eluted peaks. Mass chromatography has the

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

advantage over SIM in that full spectra are available for detailed interpretation; however, the method is somewhat less sensitive than SIM.

*Gas chromatography–olfactometry*

As mentioned earlier, high resolution GC is capable of separating and detecting hundreds of volatile compounds in a single run. However, it is likely that only a small number of these compounds actually contribute to the aroma of the product. Various approaches have been taken to identify these key odourants. These include the calculation of odour activity values (OAVs), use of GC–olfactometry (GCO) and sensory analysis of aroma models (Grosch, 1993, 1994, 2001). In GCO, the analytes are first separated by the GC and then delivered, generally in parallel with an electronic detector, to an olfactometer (sniff port) where they are mixed with humidified air. Human panelists continuously evaluate (nasally) the air emitted from the olfactometer and record their perceptions, such as odour intensity and odour description of the detected compounds. GCO has been reviewed (Acree, 1993; Blank, 2002). Common methods based on GCO include aroma extract dilution analysis (AEDA) (Grosch, 1993, 1994), CharmAnalysis (Acree, 1993), Osme (McDaniel *et al.*, 1990), nasal impact frequency (NIF, Pollien *et al.*, 1997; SNIF, Chaintreau, 2002) and post-peak intensity scaling (Avsar *et al.*, 2004). These methods differ mainly in how the GCO data are recorded and analysed. All GCO methods should be considered as screening methods since they do not provide an absolute measure of odour potency. Instead, GCO data should be used to indicate odour-active volatiles for subsequent quantitative analysis and sensory studies. Among the various GCO methods available, the dilution techniques have been most often used in the analysis of milk flavour.

GCO has been applied for aroma characterization of fresh (Moio *et al.*, 1993; Iwatsuki *et al.*, 1999; Bendall, 2001), pasteurized and UHT milks (Moio *et al.*, 1994; Iwatsuki *et al.*, 1999), milk with light-induced flavour (Cadwallader and Howard, 1998) and nonfat dry milk (Karagül-Yüceer *et al.*, 2001; Carunchia Whetstine and Drake, 2007). Tables 7.1 and 7.2 provide listings of characteristic aroma components identified in fluid and dried milk products by GCO. Two reviews of the application of GCO for the analysis of dairy products have also been published (Friedrich and Acree, 1998; Parliment and McGorrin, 2000).

*Quantitative analysis*

The ideal quantitative analysis procedure should have high precision and accuracy. Internal standard methodology has been the preferred method for routine analysis of volatile compounds in cheese products. In the internal standard method, known amounts of surrogate internal standards that mimic the analytes of interest as much as possible are added to the sample matrix prior to performing the volatile isolation procedure. The internal standards compensate for variations in extraction procedures, injection volume and detector drift. Internal standards should possess chemical, spectral and chromatographic properties that are similar to the compounds being analysed. They should be

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

readily available, stable, inexpensive, of high (or known) purity, and must not interfere with the analytes during GC. For highest accuracy it is important to determine the relative recoveries and GC response factors for the internal standards relative to the target analytes. These recovery factors may be based on GC-MS (full scan, SIM, or mass chromatography) or use of a selective detector (e.g. flame photometric detector for selective analysis of sulphur-containing compounds).

The perfect internal standard is an isotopic analogue of the analyte of interest. This method is called stable isotope dilution analysis (SIDA) and involves the use of stable isotopes (deuterium or carbon-13 labelled) as internal standards. GC-MS analysis (by either electron-impact-MS or chemical-ionization-MS) is required, since the isotopic internal standard co-elutes with the analyte but is resolvable by MS. The method is self-correcting for recovery losses during sample work-up and chromatography, since the labelled and unlabelled compounds have essentially the same physical and chemical properties. The main disadvantage of SIDA is the cost associated with the synthesis of the isotopic analogues. SIDA has been used in the analysis of milk aroma constituents (Bendall and Olney, 2001; Preininger and Ullrich, 2001; Czerny and Schieberle, 2007).

*Application of multivariate analysis techniques and electronic noses*

Chemometrics/multivariate analysis (MVA) techniques can be applied for the interpretation of complex instrumental data for three main purposes: (1) to explore patterns of association in datasets, (2) to continuously monitor properties of materials, and (3) to classify materials (Marsili, 2007b). While researchers have applied MVA techniques to understand the effect of production, processing and storage on milk flavour volatiles (Contarini *et al.*, 1997; Contarini and Povolo, 2002; Fernandez *et al.*, 2003; Vazquez-Landaverde *et al.*, 2006), this approach is most commonly used for the analysis of data from electronic nose (e-nose) instruments which utilize sensory arrays to obtain multiple responses for vapour (e.g., headspace) samples. Some commercial electronic nose devices make use of conducting polymer or metal oxide sensor arrays (Harper, 2001; Pearce *et al.*, 2003). These instruments have experienced limited success due to sensor drift, need for frequent calibration and other factors (Marsili, 2002a). Nowadays, the mass spectrometric detector (MSD)-based e-noses are more popular since they overcome many of the problems of the sensor array-based instruments (Marsili, 2002a).

Electronic noses are effective for discriminating different kinds of milk (Visser and Taylor, 1998; Brudzewski *et al.*, 2004; Yu *et al.*, 2007) and can be used to predict shelf-life (Labreche *et al.*, 2005). Korel and Balaban (2002) were able to correlate sensory data for milk with microbial loads using an electron nose equipped with 12 conducting polymer sensors. Meanwhile, Capone *et al.* (2001) demonstrated that an electronic nose based on semiconductor thin films could distinguish between UHT and pasteurized milk.

MS-based electronic noses have been successfully used to detect specific flavour defects in milk, such as trimethylamine (Ampuero *et al.*, 2002), for

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

differentiation of normal-tasting milk from those containing off-flavours from light, heat, copper abuse or from microbial contamination (Marsili, 1999), and for shelf-life prediction (Marsili, 2000).

## 7.4 Measurement of colour

Realizing the importance of colour, dairy researchers early on developed a number of methodologies to measure the colour attributes of milk products. The first attempts at measuring the colour of fluid milk involved the use of crude colorimeters (Doan, 1924). The Munsell colour system was later used to measure degree of browning by the visual comparison of solutions containing extracted brown pigments to colour references (Webb and Holm, 1930). Degree of browning was also estimated using spectrophotometric methods based on the reflectance properties of the unaltered product (Kass and Palmer, 1940; Nelson, 1948; Burton, 1954), based on the transmittance properties of solutions containing extracted pigments (Choi *et al.*, 1949; Tinkler *et al.*, 1955), and by absorption of 2-thiobarbituric acid reactive products (of 5-hydroxyfurfural) (Keeney and Bassette, 1959). Browning has also been estimated by absorption at 420 nm (Olano and Martínez-Castro, 1996).

Today, the measurement of colour can be considered a mature science (MacDougall, 2002; Francis, 2003). This is due to the development and commercialization of dedicated colour measurement devices, such as trichromatic (or tristimulus) colorimeters in the mid-1950s (Hunter, 1958) and, more recently, spectrophotometer-based systems (Francis, 2003). These systems provide a rapid and convenient means for the routine analysis of food colour (MacDougall, 2002; Francis, 2003). Since 1960, nearly all of the studies conducted on milk colour have made use of tristimulus colorimeters or spectophotometers (Pagliarini *et al.*, 1990; Hardy and Fanni, 1981; Rampilli and Andreini, 1992; Popov-Raljić *et al.*, 2008). Hunter (CIE) L a b and CIELAB L a* b* are two commonly used systems for the analysis of colorimeter data, where $L$ = lightness or darkness, +a = redness, −a = greenness, +b = yellowness and −b = blueness (Francis, 2003). The L a b values are often transformed into colour difference values ($\Delta E$) for comparison between any two samples using the following equation:

$$\Delta E = [\Delta L^2 + \Delta a^2 + \Delta b^2]^{0.5}$$

Since the whiteness of milk products is often the most critical colour attribute, some researchers have also used the variation in lightness value ($\Delta L$) as a measure of degree of difference in whiteness compared to a white standard (Hardy and Fanni, 1981). Some instruments are capable of transforming the data into whiteness values (WI) (Gervilla *et al.*, 2001). Yellow index (YI) was used by Pagliarini *et al.* (1990) to monitor the colour changes in heat-treated milk. In addition to the above-mentioned tristimulus systems, spectral reflectance measurements (400 to 700 nm) are also a suitable estimate of degree of

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

whiteness in milk (Nelson, 1948; Quiñones *et al.*, 1997; Solah *et al.*, 2007). Some recent applications of colorimetric and spectrophotometric systems for measurement of colour in milk products can be found in the published literature (Nielsen *et al.*, 1997; Browning *et al.*, 2001; Gervilla *et al.*, 2001; Nozière *et al.*, 2006; Solah *et al.*, 2007; Popov-Raljić *et al.*, 2008).

## 7.5 Future trends

The understanding of how certain variables, such as production, processing and storage, impact the flavour and colour of milk will continue to interest dairy scientists. Measurement of milk colour using colorimeters and spectrophotometers is a mature science and little development in this field is expected to occur in the near future. On the other hand, techniques used for flavour measurement of milk products are constantly evolving. Such methods include the difficult and labour-intensive GCO and GC-MS methods, which allow for detailed aroma characterization. In addition, relatively simple and easy to use methods, including SHA, DHA and HS-SPME and electronic nose technology, provide useful, albeit somewhat limited, information about how the volatile constituents of milk are impacted by new and emerging dairy processing techniques, as well as other factors such as packaging and storage.

## 7.6 Sources of further information and advice

Texts that provide more detailed information about new and emerging methods for measuring volatile compounds in foods are highly recommended (Reneiccius, 2006; Marsili, 2007a). For the latest developments in flavour research the reader should consult scientific journals, proceedings (symposia) of scientific meetings and technical papers presented at scientific meetings.

IP Address: 129.132.208.100

## 7.7 References

ACREE T E (1993), 'Gas chromatography–olfactometry', in Ho C-T and Manley C H, *Flavor Measurement*, New York, Marcel Dekker, 77–94.

ADDA J (1986), 'Flavour of dairy products', in Birch G G and Lindley M G, *Developments in Food Flavours*, London, Elsevier Applied Science Publishers, 151–172.

AMPUERO S and BOSSET J O (2003), 'The electronic nose applied to dairy products: a review', *Sensors and Actuators B*, 94, 1–12.

AMPUERO S, ZESIGER T, GUSTAFSSON V, LUNDÉN A and BOSSET J O (2002), 'Determination of trimethylamine in milk using an MS electronic nose', *Eur Food Res Technol*, 214, 163–167.

ANDERSON K and LINGNERT H (1998), 'Influence of oxygen concentration and light on the oxidative stability of cream powder', *Lebesm-Wiss u-Technol*, 31, 169–176.

ARNOLD R G, LIBBEY L M and DAY E A (1966), 'Identification of components in the stale flavor fraction of sterilized concentrated milk', *J Food Sci*, 31, 566–573.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

AVSAR Y K, KARAGÜL-YÜCEER Y, DRAKE M A, SINGH T K, YOON Y and CADWALLADER K R (2004), 'Characterization of nutty flavor in Cheddar cheese', *J Dairy Sci*, 87, 1999–2010.

AZZARA C D and CAMPBELL L B (1992), 'Off-flavours of dairy products', in Charalambous G, *Off-flavours in Foods and Beverages*, Amsterdam, Elsevier, 329–374.

BADINGS H T (1991), 'Milk', in Maarse H, *Volatile Compounds in Foods and Beverages*, New York, Marcel Dekker, 91–106.

BADINGS H T and NEETER R (1980), 'Recent advances in the study of aroma compounds of milk and dairy products', *Neth Milk Dairy J*, 34, 9–30.

BALTUSSEN E, SANDRA P, DAVID F and CRAMERS C A (1999), 'Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles', *J Mirocolumn Sep*, 11, 737–747.

BARREFORS P, GRANELLI K, APPELQVIST L-A and BJOERCK L (1995), 'Chemical characterization of raw milk samples with and without oxidative off-flavor', *J Dairy Sci*, 78, 2691–2699.

BENDALL J G (2001), 'Aroma compounds of fresh milk from New Zealand cows fed different diets', *J Agric Food Chem*, 49, 4825–4832.

BENDALL J G and OLNEY S D (2001), 'Hept-*cis*-4-enal: analysis and flavour contribution to fresh milk', *Int Dairy J*, 11, 855–864.

BICCHI C, CORDERO C, LIBERTO E, RUBIOLO P and SGORBINI B (2004), 'Automated headspace solid-phase dynamic extraction to analyse the volatile fraction of food matrices', *J Chromatogr A*, 1024, 217–226.

BLANK I (2002), 'Gas chromatography–olfactometry in food aroma analysis', in Marsili R, *Flavor, Fragrance and Odor Analysis*, New York, Marcel Dekker, 297–331.

BOELRIJK A E and DE JONG C (2003), 'Relating analytical and sensory data to predict flavour quality in dairy products', in Cadwallader K R and Weenen H, *Freshness and Shelf Life of Foods*, ACS Symposium Series 836, Washington, DC, American Chemical Society, 95–107.

BROWNING E, LEWIS M and MACDOUGALL D (2001), 'Predicting safety and quality parameters for UHT-processed milks', *Int J Dairy Technol*, 54, 111–120.

BRUDZEWSKI K, OSOWSKI S and MARKIEWICZ T (2004), 'Classification of milk by means of an electronic nose and SVM neural network', *Sensors and Actuators B*, 98, 291–298.

BURTON H (1954), 'Colour changes in heated and unheated milk. I. The browning of milk on heating', *J Dairy Res*, 21, 194–203.

BURTON H (1955), 'Colour changes in heated and unheated milk. II. The whitening of milk on heating', *J Dairy Res*, 22, 74–81.

BURTON H (1956), 'The effect of heat on the colour of milk', *Int J Dairy Technol*, 9, 146–148.

BUTRYM E (1999), 'Solving GC problems using thermal desorption and thermal extraction', *LC·GC*, 17(9S), S19–S24.

CADWALLADER K R (2007), 'Measuring cheese flavour', in Weimer B C, *Improving the Flavour of Cheese*, Cambridge, Woodhead Publishing, 401–417.

CADWALLADER K R and HOWARD C L (1998), 'Analysis of aroma-active components of light-activated milk', in Mussinan C J and Morello M J, *Flavor Analysis: Developments in Isolation and Characterization*, ACS Symposium Series 705, Washington, DC, American Chemical Society, 343–358.

CADWALLADER K R and SINGH T K (2009), 'Flavours and off-flavours in milk and dairy products', in McSweeny P L H and Fox P F, *Advanced Dairy Chemistry* – 3.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

*Lactose, Water, Salts and Minor Constituents*, New York, Springer Science+ Business Media, 631–690.

CAPONE S, EPIFANI M, QUARANTA F, SICILIANO P, TAURINO A and VASANELLI L (2001), 'Monitoring of rancidity of milk by means of an electronic nose and a dynamic PCA analysis', *Sensors and Actuators B*, 78, 174–179.

CARUNCHIA WHETSTINE M E and DRAKE M A (2007), 'The flavor and flavor stability of skim and whole milk powders', in Cadwallader K R, Drake M A and McGorrin R J, *Flavor of Dairy Products*, ACS Symposium Series 971, Washington, DC, American Chemical Society, 217–251.

CARUNCHIA WHETSTINE M E, CADWALLADER K R and DRAKE M A (2005), 'Characterization of aroma compounds responsible for the rosy/floral flavor in Cheddar cheese', *J Agric Food Chem*, 53, 3126–3132.

CARUNCHIA WHETSTINE M E, DRAKE M A, NELSON B K and BARBANO D M (2006), 'Flavor profiles of full-fat and reduced-fat cheese and cheese fat made from aged Cheddar with the fat removed using a novel process', *J Dairy Sci*, 89, 505–517.

CHAINTREAU A (2001), 'Simultaneous distillation–extraction: from birth to maturity – review', *Flavour Fragrance J*, 16, 136–148.

CHAINTREAU A (2002), 'Quantitative use of gas chromatography–olfactometry: The GC-"SNIF" method', in Marsili R, *Flavor, Fragrance and Odor Analysis*, New York, Marcel Dekker, 333–348.

CHOI R P, KONCUS A F, O'MALLEY C M and FAIRBANKS B W (1949), 'A proposed method for the determination of color of dry products of milk', *J Dairy Sci*, 32, 580–586.

CHRIST I, KUEHN U B and STRASSBURGER K (2007), 'Solid phase dynamic extraction: A technique for extracting more analytes from samples', in Marsili R, *Sensory-directed Flavor Analysis,* Boca Raton, FL, Taylor & Francis, 155–180.

CHRISTENSEN K R and REINECCIUS G A (1992), 'Gas chromatographic analysis of volatile sulfur compounds from heated milk using static headspace sampling', *J Dairy Sci*, 75, 2098–2104.

COLAHAN-SEDERSTROM P M and PETERSON D G (2005), 'Inhibition of key aroma compounds generated during ultrahigh-temperature processing of bovine milk via epicatechin addition', *J Agric Food Chem*, 53, 398–402.

COLE A and WOOLFENDEN E (1992), 'Gas extraction techniques for sample preparation in gas chromatography', *LCGC*, 10, 76–82.

CONTARINI G and POVOLO M (2002), 'Volatile fraction of milk: Comparison between purge and trap and solid phase microextraction techniques', *J Agric Food Chem*, 50, 7350–7355.

CONTARINI G, POVOLO M, LEARDI R and TOPPINO P M (1997), 'Influence of heat treatment on the volatile compounds of milk', *J Agric Food Chem*, 45, 3171–3177.

CORFIELD A (1955), 'Bacterial fishiness in milk', *Dairy Industries,* 1035–1037.

CZERNY M and SCHIEBERLE P (2007), 'Influence of polyethylene packaging on the adsorption of odour-active compounds from UHT-milk', *Eur Food Res Technol*, 225, 215–223.

DAY E A, LILLARD D A and MONTGOMERY M W (1963), 'Auto oxidation of milk lipids: III. Effect on the flavor of the additive interactions of carbonyl compounds of sub-threshold concentrations', *J Dairy Sci*, 46, 291–294.

DOAN F J (1924), 'The color of cow's milk and its nutritive value', *J Dairy Sci*, 7, 146–153.

DRAKE M A, MIRACLE R E, CAUDLE A D and CADWALLADER K R (2007), 'Relating sensory and instrumental analysis', in Marsili R, *Sensory-directed Flavor Analysis*, Boca

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

Raton, FL, CRC, Taylor & Francis, 23–53.

ENGEL W, BAHR W and SCHIEBERLE P (1999), 'Solvent assisted flavor evaporation – a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices', *Eur Food Res Technol*, 209, 237–241.

ETIÉVANT P X (1996), 'Artifacts and contaminants in the analysis of food flavor', *CRC Crit Rev Food Sci Nutr*, 36, 733–745.

FERNANDEZ C, ASTIER C, ROCK E, COULON J-B and BERDAQUÉ J-L (2003), 'Characterization of milk by analysis of its terpene fractions', *Int J Food Sci Technol*, 38, 445–451.

FORSS D A (1979), 'Review of the progress of dairy science: Mechanisms of formation of aroma compounds in milk and milk products', *J Dairy Sci*, 46, 691–706.

FORSS D A and SUGISAWA H (1981), 'Brief reviews of dairy and soy products', in Teranishi R and Flath R A, *Flavor Research*, New York, Marcel Dekker, 355–372.

FORSS D A, PONT E G and STARK W J (1955a), 'The volatile compounds associated with oxidized flavor in skim milk', *J Dairy Res*, 22, 91–95.

FORSS D A, PONT E G and STARK W J (1955b), 'The further observations on the volatile compounds associated with oxidized flavour in skim milk', *J Dairy Res*, 22, 345–351.

FRANCIS F J (2003), 'Color analysis', in Neilson S S, *Food Analysis*, 3rd edn, New York, Springer Science+Business Media, 529–541.

FRIEDRICH J E and ACREE T E (1998), 'Gas chromatography olfactometry (GC/O) of dairy products', *Int Dairy J*, 8, 235–241.

GERVILLA R, FERRAGUT V and GUAMIS B (2001), 'High hydrostatic pressure effects on color and milk-fat globule of ewe's milk', *J Food Sci*, 66, 880–885.

GRANELLI K, BARREFORS P, BJÖRCK L and APPELQVIST L-Å (1998), 'Further studies on lipid composition of bovine milk in relation to spontaneous oxidised flavour', *J Sci Food Agric*, 77, 161–171.

GROSCH W (1993), 'Detection of potent odorants in foods by aroma extract dilution analysis', *Trends Food Sci Technol*, 4, 68–73.

GROSCH W (1994), 'Determination of potent odourants in foods by aroma extract dilution analysis (AEDA) and calculation of odour activity values (OAVs)', *Flavour Fragrance J*, 9, 147–158.

GROSCH W (2001), 'Evaluation of the key odorants of foods by dilution experiments, aroma models and omission', *Chem Senses*, 26, 533–545.

HAMMOND E G and SEALS R G (1972), 'Oxidized flavor in milk and its simulation', *J Dairy Sci*, 55, 1567–1569.

HARDY J and FANNI J (1981), 'Application of reflection photometry to the measurement of milk coagulation', *J Food Sci*, 46, 1956–1957.

HARMON A D (2002), 'Solid-phase microextraction for the analysis of aromas and flavors', in Marsili R, *Flavor, Fragrance and Odor Analysis*, New York, Marcel Dekker, 75–106.

HARPER J (2001), 'The strengths and weaknesses of the electronic nose', in Rouseff R and Cadwallader K R, *Headspace Analysis of Foods and Flavors*, New York, Kluwer Academic/Plenum Publishers, 59–71.

HARTMAN T G, LECH J, KARMAS K, SALINAS J, ROSEN R T and HO C-T (1993), 'Flavor characterization using adsorbent trapping–thermal desorption or direct thermal desorption–gas chromatography and gas chromatography–mass spectrometry', in Ho C-T and Manley C H, *Flavor Measurement*, New York, Marcel Dekker, 37–60.

HEDEGAARD R V, KRISTENSEN D, NIELSEN J H, FRØST M B, ØSTDAL H, HERMANSEN J E, KRÖGER-OHLSEN M and SKIBSTED L H (2006), 'Comparison of descriptive sensory analysis

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

and chemical analysis for oxidative changes in milk', *J Dairy Sci*, 89, 495–504.
HINSHAW J V (1990), 'Headspace sampling', *LCGC*, 8, 362–368.
HUMPHRISS E (1953), 'Chromogenic bacteria and milk', *Dairy Industries*, 781–782.
HUNTER R S (1958), 'Photoelectric color difference meter', *J Optical Soc America*, 48, 985–995.
IMHOF R and BOSSET J O (1994), 'Quantitative GC-MS analysis of volatile flavour compounds in pasteurized milk and fermented milk products applying a standard addition method', *Lebesm-Wiss u-Technol*, 27, 265–269.
IWATSUKI K, MIZOTA Y, KUBOTA T, NISHIMURA O, MASUDA H, SOTOYAMA K and TOMITA M (1999), 'Evaluation of aroma of pasteurized and UHT processed milk by aroma extract dilution analysis', *Nippon Shokuhin Kagaku Kogaku Kaishi*, 46, 587–597.
JENG W, BASSETTE R and CRANG R E (1988), 'Effects of light and copper ions on volatile aldehydes of milk and milk fractions', *J Dairy Sci*, 71, 2366–2372.
JUNG M Y, YOON S H, LEE H O and MIN D B (1998), 'Singlet oxygen and ascorbic acid effects on the dimethyl disulfide and off-flavor formation in skim milk exposed to light', *J Food Sci*, 63, 408–412.
KARAGÜL-YÜCEER Y, DRAKE M A and CADWALLADER K R (2001), 'Aroma-active components of nonfat dry milk', *J Agric Food Chem*, 49, 2948–2953.
KARAGÜL-YÜCEER Y, CADWALLADER K R and DRAKE M A (2002), 'Volatile flavor components of stored nonfat dry milk', *J Agric Food Chem*, 50, 305–312.
KARAGÜL-YÜCEER Y, DRAKE M A and CADWALLADER K R (2003), 'Aroma characterization of fresh and stored-nonfat dry milk, in Cadwallader K R and Weenen H, *Freshness and Shelf Life of Foods*, ACS Symposium Series 836, Washington, DC, American Chemical Society, 108–123.
KASS J P and PALMER L S (1940), 'Browning of autoclaved milk, chemical factors involved', *Ind Eng Chem*, 32, 1360–1366.
KEENEY M and BASSETTE R (1959), 'Detection of intermediate compounds in the early stages of browning reaction in milk products', *J Dairy Sci*, 42, 945–960.
KESSLER H-G and FINK R (1986), 'Changes in heated and stored milk with an interpretation by reaction kinetics', *J Food Sci*, 51, 1105–1111, 1155.
KIM Y D and MORR C V (1996), 'Dynamic headspace analysis of light activated flavor in milk', *Int Dairy J*, 6, 185–193.
KOREL F and BALABAN M O (2002), 'Microbial and sensory assessment of milk with an electronic nose', *J Food Sci*, 67, 758–764.
LABRECHE S, BAZZO S, CADE S and CHANIE E (2005), 'Shelf life determination by electronic nose: application to milk', *Sensors and Actuators B*, 106, 199–206.
LUNDEN A, GUSTAFSSON V, IMHOF M, GAUCH R and BOSSET J O (2002), 'High trimethylamine concentration in milk from cows on standard diets is expressed as fishy off-flavour', *J Dairy Res*, 69, 383–390.
MACDOUGALL D B (2002), 'Colour measurement of food', in MacDougall D B, *Colour in Food: Improving Food Quality*, Cambridge, Woodhead Publishing, 32–63.
MAIRACA R and BOSSET J O (1997), 'Instrumental analysis of volatile (flavour) compounds in milk and dairy products (a review)', *Lait*, 77, 13–40.
MARSILI R T (1999), 'SPME-MS-MVA as an electronic nose for the study of off-flavors in milk', *J Agric Food Chem*, 47, 648–654.
MARSILI R T (2000), 'Shelf-life prediction of processed milk by solid-phase microextraction, mass spectrometry, and multivariate analysis', *J Agric Food Chem*, 48, 3470–3475.
MARSILI R T (2002a), 'Combining mass spectrometry and multivariate analysis to make a

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

reliable and versatile electronic nose', in Marsili R, *Flavor, Fragrance, and Odor Analysis*, New York, Marcel Dekker, 349–374.

MARSILI R (2002b), 'SPME comparison studies and what they reveal', in Marsili R, *Flavor, Fragrance and Odor Analysis*, New York, Marcel Dekker, 205–248.

MARSILI R T (2003), 'Flavours and off-flavours in dairy foods', in Roginski H, Fuquay J W and Fox P F, *Encyclopedia of Dairy Science*, London, Academic Press, 1069–1081.

MARSILI R (2007a), *Sensory-directed Flavor Analysis*, Boca Raton, FL, Taylor & Francis.

MARSILI R T (2007b), 'The application of chemometrics for studying flavor and off-flavor problems in foods and beverages', in Marsili R, *Sensory-directed Flavor Analysis*, Boca Raton, FL, Taylor & Francis, 181–204.

MARSILI R T and MILLER N (1998), 'Determination of the cause of off-flavours in milk by dynamic headspace GC/MS and multivariate data analysis', in Mussinan C, Contis E, Ho C-T, Parliament T, Spanier A and Shahidi F, *Food Flavor Formation, Analysis, and Packaging Influences*, Amsterdam, Elsevier, 159–171.

MCDANIEL M R, MIRANDA-LÓPEZ R, WATSON B T, MICHAELS N J and LIBBEY L M (1990), 'Pinot noir aroma: a sensory/gas chromatographic approach', in Charalambous G, *Flavors and Off-flavors*, Amsterdam, Elsevier, 23–36.

MCGORRIN R J (2007), 'Flavor analysis of dairy products', in Cadwallader K R, Drake M A and McGorrin R J, *Flavor of Dairy Products*, ACS Symposium Series 971, Washington, DC, American Chemical Society, 23–49.

MEHTA R S and BASSETTE R (1979), 'Volatile compounds in UHT-sterilized milk during fluorescent light exposure and storage in the dark', *J Food Protection*, 42, 256–258.

MOIO L, LANGLOIS D, ETIÉVANT P and ADDEO F (1993), 'Powerful odorants in bovine, ovine, caprine and water buffalo milk determined by means of gas chromatography-olfactometry', *J Dairy Sci*, 60, 215–222.

MOIO L, ETIÉVANT P, LANGLOIS D, DEKIMPE J and ADDEO F (1994), 'Detection of powerful odorants in heated milk by use of extract dilution sniffing analysis', *J Dairy Res*, 61, 385–394.

MOUNCHILI A, WICHTEL J J, BOSSET J O, DOHOO I R, IMHOF M, ALTIERI D, MALLIA S and STRYHN H (2005), 'HS-SPME gas chromatographic characterization of volatile compounds in milk tainted with off-flavour', *Int Dairy J*, 15, 1203–1215.

NELSON V (1948), 'The spectrophotometric detection of the color of milk', *J Dairy Sci*, 31, 409–414.

NIELSEN B R, STAPELFELDT H and SKIBSTED L H (1997), 'Early prediction of shelflife of medium-heat whole milk powders using stepwise multiple regression and principal component analysis', *Int Dairy J*, 7, 341–348.

NOZIÈRE P, GROLLER P, DURAND D, FERLAY A, PRADEL P and MARTIN B (2006), 'Variations in carotenoids, fat-soluble micronutrients, and color in cow's plasma and milk following changes in forage and feeding level', *J Dairy Sci*, 89, 2634–2648.

NURSTEN H E (1997), 'The flavour of milk and dairy products: I. Milk of different kinds, milk powder, butter and cream', *Int J Dairy Technol*, 50, 48–56.

OLANO A and MARTÍNEZ-CASTRO I (1996), 'Nonenzymatic browning', in Nollet L M (ed.), *Handbook of Food Analysis*, Vol, 2, New York, Marcel Dekker, 1683–1721.

OTT A, FAY L B and CHAINTREAU A (1997), 'Determination and origin of the aroma impact compounds of yogurt flavor', *J Agric Food Chem*, 45, 850–858.

PAGLIARINI E, VERNILLE M and PERI C (1990), 'Kinetic study on color changes in milk due to heat', *J Food Sci*, 55, 1766–1767.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

PARK P S W and GOINS R E (1992), 'Determination of volatile lipid oxidation products by dynamic headspace–capillary gas chromatographic analysis with application to milk-based nutrition products', *J Agric Food Chem*, 40, 1581–1585.

PARK R J (1969), 'Weed taints in dairy produce. I. Lepidium taint', *J Dairy Res*, 36, 31–36.

PARK R J, ARMITT J D and STAR D P (1969), 'Weed taints in dairy produce. II. Coronopus or land cress taint in milk', *J Dairy Res*, 36, 37–46.

PARKS O W, SCHWARTZ D P and KEENEY M (1964), 'Identification of *o*-aminoacetophenone as a flavour compound in stale dry milk', *Nature*, 202, 185–187.

PARKS O W, WONG N P, ALLEN C A and SCHWARTZ D P (1969), '6-*trans*-Nonenal: An off-flavor component of foam-spray-dried milks', *J Dairy Sci*, 52, 953–956.

PARLIMENT T H (2002), 'Solvent extraction and distillation techniques', in Marsili R, *Flavor, Fragrance, and Odor Analysis*, New York, Marcel Dekker, 1–23.

PARLIMENT T H and MCGORRIN R J (2000), 'Critical flavour compounds in dairy products', in Risch S J and Ho C T, *Flavour Chemistry: Industrial and Academic Research*, ACS Symposium Series 756, Washington, DC, American Chemical Society, 44–71.

PATTON S (1955), 'Browning and associated changes in milk and its products: a review', *J Dairy Sci*, 38, 457–478.

PEARCE T C, SCHIFFMAN S S, NAGLE H T and GARDNER J W (2003), *Handbook of Machine Olfaction*, Weinheim, Germany, Wiley-VCH.

PERKINS M L, D'ARCY B R, LISLE A T and DEETH H (2005), 'Solid phase microextraction of stale flavour volatiles from the headspace of UHT milk', *J Sci Food Agric*, 85, 2421–2428.

POLINENI R V and PETERSON D G (2005), 'Influence of thermal processing conditions on flavor stability in fluid milk: Benzaldehyde', *J Dairy Sci*, 88, 1–6.

POLLIEN P, OTT A, MONTIGON F, BAUMGARTNER M, MUÑOS-BOX R and CHAINTREAU A (1997), 'Hyphenated headspace-gas chromatography–sniffing technique: screening of impact odorants and quantitative aromagram comparisons', *J Agric Food Chem*, 45, 2630–2637.

POPOV-RALJIĆ J V, LAKIĆ N S, LALIĆIĆ-PETRONIJEVIĆ J G, BARAĆ M B and SIKIMIĆ V M (2008), 'Color changes of UHT milk during storage', *Sensors*, 8, 5961–5974.

PREININGER M and ULLRICH F (2001), 'Trace compound analysis for off-flavor characterization of micormilled milk powder', in Leland J V, Schieberle P, Buettner A and Acree T E, *Gas Chromatography-Olfactometry: The State of the Art*, ACS Symposium Series 782, Washington, DC, American Chemical Society, 46–61.

QUIÑONES H J, BARBANO D M and PHILLIPS L G (1997), 'Influence of protein standardization by ultrafiltration on the viscosity, color, and sensory properties of skim and 1% milk', *J Dairy Sci*, 80, 3142–3151.

RAMPILLI M and ANDREINI R (1992), 'Evaulation of colour components in sterilized milk', *Ital J Food Sci*, 4, 285–291.

REINECCIUS G (2006), *Flavor Chemistry and Technology,* 2nd edn, New York, Taylor & Francis.

RHIM J W, JONES V A and SWARTZEK K (1988), 'Initial whitening phenomenon of skim milk on heating', *Lebensm-Wiss u-Technol*, 21, 339–341.

ROBERTS D D, POLLIEN P and MILO C (2000), 'Solid-phase microextraction method development for headspace analysis of volatile flavor compounds', *J Agric Food Chem*, 48, 2430–2437.

RYSSTAD G, EBBESEN A and EGGESTAD J (1998), 'Sensory and chemical quality of UHT-milk stored in paperboard cartons with different oxygen and light barriers', *Food Additives and Contaminants*, 15, 112–122.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

SCHLICHTHERLE-CERNY H, IMHOF M I, FERNÁNDEZ GARCÍA E and BOSSET J O (2007), 'From pasture to cheese: Changes in terpene composition', in Cadwallader K R, Drake M A and McGorrin R J, *Flavor of Dairy Products*, ACS Symposium Series 971, Washington, DC, American Chemical Society, 123–133.

SEÑORANS F J, TABERA J, HERRAIZ M and REGLERO G (1996), 'A method for the direct isolation and gas chromatographic analysis of milk flavor components using a programmed temperature vaporizer', *J Dairy Sci*, 79, 1706–1712.

SINGH H and CREAMER L K (1992), 'Heat stability of milk', in Fox P F (ed.), *Advanced Dairy Chemistry, Proteins, 1*, Cambridge, Elsevier, pp. 621–656.

SINGH T K, DRAKE M A and CADWALLADER K R (2003), 'Flavor of Cheddar cheese: a chemical and sensory perspective', *Comprehensive Reviews Food Science and Food Safety*, 2, 139–162.

SINGH T K, CADWALLADER K R and DRAKE M A (2007), 'Biochemical processes in the production of flavour in milk and milk products', in Hui Y H, *Handbook of Food Products Manufacturing*, Volume 2, Hoboken, NJ, John Wiley & Sons, 715–748.

SOLAH V A, STAINES V, HONDA S and LIMLEY H A (2007), 'Measurement of milk color and composition: effect of dietary intervention on Western Australian Holstein-Friesian cow's milk quality', *J Food Sci*, 72, S560–S566.

SOLANO-LOPEZ C E, JI T and ALVAREZ V B (2005), 'Volatile compounds and chemical changes in ultrapasteurized milk packaged in polyethylene terephthalate containers', *J Food Sci*, 70, C407–C412.

TINKLER F H, STRIBLEY R C and BERNHART F W (1955), 'Improvements in a method for determination of the color of milk and milk products', *J Dairy Sci*, 38, 634–639.

TOSO B, PROCIDA G and STEFANON B (2002), 'Determination of volatile compounds in cows' milk using headspace GC-MS', *J Dairy Res*, 69, 569–577.

VALERO E, SANZ J and MARTÍNEZ-CASTRO I (1999), 'Volatile components in microwave- and conventionally-heated milk', *Food Chem*, 66, 333–338.

VALERO E, VILLAMIEL M, MIRALLES B, SANZ J and MARTÍNEZ-CASTRO I (2001), 'Changes in flavour and volatile components during storage of whole and skimmed UHT milk', *Food Chem*, 72, 51–58.

VALLEJO-CORDOBA B and NAKAI S (1993), 'Using a simultaneous factor optimization approach for the detection of volatiles in milk by dynamic headspace gas chromatographic analysis', *J Agric Food Chem*, 41, 2378–2384.

VAN AARDT M, DUNCAN S E, MARCY J E, LONG T E, O'KEEFE S F and NIELSEN-SIMS S R (2005), 'Effect of antioxidant ($\alpha$-tocopherol and ascorbic acid) fortification of light-induced flavor of milk', *J Dairy Sci*, 88, 872–880.

VAN BOEKEL M A J S (1998), 'Effect of heating on Maillard reactions in milk', *Food Chem*, 62, 403–414.

VAS G and VÉKEY K (2004), 'Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis', *J Mass Spectrometry*, 39, 233–254.

VAZQUEZ-LANDAVERDE P A, VELAZQUEZ G, TORRES J A and QIAN M C (2005), 'Quantitative determination of thermally derived off-flavor compounds in milk using solid-phase microextraction and gas chromatography', *J Dairy Sci*, 88, 3764–3772.

VAZQUEZ-LANDAVERDE P A, TORRES J A and QIAN M C (2006), 'Effect of high-pressure-moderate-temperature processing on the volatile profile of milk', *J Agric Food Chem*, 54, 9184–9192.

VAZQUEZ-LANDAVERDE P A, QIAN M C and TORRES J A (2007), 'Kinetic analysis of volatile formation in milk subjected to pressure-assisted thermal treatments', *J Food Sci*, 72, E389–E398.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

VISSER F R and TAYLOR M (1998), 'Improved performance of the Aromascan A32S electronic nose and its potential for detecting aroma differences in dairy products', *J Sensory Studies*, 13, 95–120.

WAMPLER T P (2002), 'Analysis of food volatiles using headspace-gas chromatographic techniques', in Marsili R, *Flavor, Fragrance, and Odor Analysis*, New York, Marcel Dekker, 25–54.

WEBB B H and HOLM G (1930), 'Color of evaporated milks', *J Dairy Sci*, 13, 25–39.

WELLNITZ-RUEN W, REINECCIUS G A and THOMAS E L (1982), 'Analysis of the fruity off-flavor in milk using headspace concentration capillary column gas chromatography', *J Agric Food Chem*, 30, 512–514.

WERKHOFF P, BRENNECKE S, BRETSCHNEIDER W and BERTRAM H-J (2002), 'Modern methods for isolating and quantifying volatile flavor and fragrance compounds', in Marsili R, *Flavor, Fragrance and Odor Analysis*, New York, Marcel Dekker, 139–204.

WHITFIELD F B, JENSEN N and SHAW K J (2000), 'Role of *Yersinia intermedia* and *Pseudomonas putida* in development of a fruity off-flavour in pasteurized milk', *J Dairy Res*, 67, 561–569.

WOOD D C, MILLER J M and CHRIST I (2004), 'Headspace liquid microextraction', *LC·GC*, 22, 516–522.

YU H, WANG J and XU Y (2007), 'Identification of adulterated milk using electronic nose', *Sensors and Materials*, 19, 275–285.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

# 8

# Analysing and improving the mineral content of milk

**F. Gaucheron, INRA – Agrocampus Ouest, France**

**Abstract:** Minerals of milk correspond to calcium, magnesium, sodium, potassium, inorganic phosphate, chloride and citrate. These ions are distributed between the aqueous and micellar phases of milk. The chapter first discusses the composition and repartition (named salt equilibria) of these ions in milk. It then describes sample preparation and some analytical methods to determine their content and repartition in milk. In the last part, the ways to modulate (increase or decrease) the content of major minerals by addition of different salts or reduction by different technologies such as membrane filtration or chromatography are described.

**Key words:** milk, calcium, salt equilibria, casein micelles, analyses.

IP Address: 129.132.208.100

## 8.1 The minerals of milk

Compared with proteins or lipids of milk, the mineral fraction is quantitatively low. It contains about 8–9 g/l of matter and is composed of cations (calcium, magnesium, sodium and potassium) and anions (inorganic phosphate, citrate and chloride). In milk, these ions are more or less associated between themselves and with proteins. Depending on the type of ion, they are present in the aqueous phase (in the case of sodium, potassium and chloride) or partially associated with casein molecules (as is the case for calcium, magnesium, inorganic phosphate and citrate) to form casein micelles (Table 8.1).

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

**Table 8.1** Salts partition in cow's milk. The aqueous fraction corresponds to the mineral fraction diffusing through an ultrafiltration membrane with a molecular weight cut-off of 10,000 Da

| Ion | Total concentration (mM) | Aqueous concentration (mM) |
|---|---|---|
| Ca | 30.0 | 9.0 |
| Pi | 21.0 | 11.0 |
| Mg | 5.0 | 3.5 |
| Citrate | 9.0 | 8.1 |
| Na | 22.0 | 0.5 |
| K | 35.0 | 0.7 |
| Cl | 30.0 | 0.0 |

### 8.1.1 Minerals in the aqueous phase

In the aqueous phase, ions are free (ionic form) or associated to form salts (Table 8.2). Quantitatively, the associations between cations and anions depend on the values of the different association constants (or affinity) and the solubilities of the salts. Globally, calcium exists as ionic calcium (free ion) and is associated with citrate (as the trivalent citrate $Cit^{3-}$), and to a lesser degree with inorganic phosphate (as a mixture of $H_2PO_4^-$ and $HPO_4^{2-}$) and chloride. The low quantity of calcium phosphate in the aqueous fraction is due to its low solubility. Sodium and potassium exist mainly as free ions but a small part of these ions is associated with citrate, inorganic phosphate and chloride. Taking account of these different associations, the aqueous phase of milk at pH 6.6–6.7 appears to be supersaturated in calcium phosphate and has an ionic strength of about 80 mM.

IP Address: 129.132.208.100

### 8.1.2 Minerals in the micellar phase

In the micellar phase, the mineral fraction is not well defined because of its heterogeneity and complexity. Schematically, it can be described as a mixture of calcium phosphate (which is an inorganic phosphate) and calcium caseinate (containing organic phosphate present in the phosphoseryl residues of $\alpha s_1$-, $\alpha s_2$-

**Table 8.2** Theoretical concentrations of ions and salts (mM) in the aqueous phase of milk at pH 6.75

| Constituent | Free | $Ca^{2+}$ | $Mg^{2+}$ | $Na^+$ | $K^+$ |
|---|---|---|---|---|---|
| $HCit^{2-}$ | 0.03 | 0.01 | 0.00 | 0.00 | 0.01 |
| $Cit^{3-}$ | 0.17 | 6.04 | 1.79 | 0.02 | 0.03 |
| $H_2PO_4^-$ | 3.65 | 0.07 | 0.02 | 0.05 | 0.08 |
| $HPO_4^{2-}$ | 3.67 | 0.60 | 0.67 | 0.51 | 0.69 |
| $Cl^-$ | 27.80 | 0.24 | 0.09 | 0.34 | 0.58 |
| Ionic form | 0.00 | 2.12 | 1.14 | 20.90 | 36.26 |

Source: Mekmene *et al.* (2009).

© Woodhead Publishing Limited, 2010

Downloaded From Vet-eBooks.com

and $\beta$-caseins). Results obtained from X-ray absorption and infrared spectroscopy indicate that the micellar calcium phosphate closely resembles brushite (Nelson *et al.*, 1989). By calculation of the apparent solubility product, a modified dicalcium phosphate $Ca(HPO_4)_{0.7}(PO_4)_{0.2}$ was proposed by Holt (1985). Using high-resolution transmission electron microscopy and diffraction methods, McGann *et al.* (1983) and Lyster *et al.* (1984) indicated an amorphous nature of the micellar calcium phosphate. The micellar calcium phosphate appeared as several distinct regions of higher electron density with an estimated diameter of about 2.5 nm. More recently, Marchin *et al.* (2007) confirmed by cryo-transmission electron microscopy and small-angle X-ray scattering/ultrasmall-angle X-ray scattering that the unique sub-structures existing in casein micelles were small granules of calcium phosphate having diameters close to 2.5 nm.

The associations between casein molecules and minerals are probably responsible for the structure and stability of casein micelles. Indeed, micellar calcium phosphate binds the negative sites present on the phosphoseryl residues and it can be considered as both a crosslinking and a neutralising agent of phosphoseryl residues. In a typical casein micelle, there are about $10^4$ polypeptide chains of casein molecules associated with about $3 \times 10^3$ microgranules of amorphous calcium phosphate. In all structural models of the casein micelle proposed, the micellar calcium phosphate is an integral part of the casein micelle. The models of Schmidt (1982) and of Holt and Horne (1996) are essentially used. Schmidt (1982) proposed a sub-unit structure linked by micellar calcium phosphate, although the sub-micelles are not considered in the model of Holt and Horne (1996). These latter authors considered caseins as rheomorphic proteins and the micellar calcium phosphate, which they called calcium phosphate nanoclusters, is mainly bound to the phosphoserine residues of casein and secondarily to carboxyl groups of glutamyl and aspartyl residues.

IP Address: 129.132.208.100

### 8.1.3 Salt equilibria

The partitions of ions and salts between the aqueous and micellar phases are in equilibria. The salt equilibria are sensitive to the physico-chemical conditions (Fig. 8.1) (references cited by De la Fuente, 1998; Gaucheron, 2004) such as variations in pH (acidification or alkalinisation), heat treatment, cooling, and

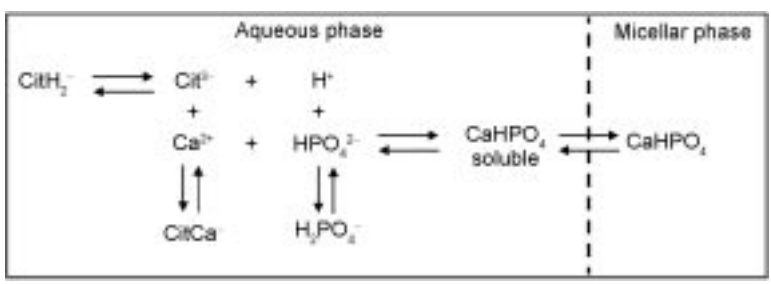



**Fig. 8.1** Salt equilibria between aqueous and micellar phases at pH 6.75. The concentrations of different mineral associations are indicated in Table 8.2.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

additions of different salts. For example, acidification, cooling, and addition of chelating agents induce a demineralisation of casein micelles. On the other hand, heat treatment or addition of calcium led to a transfer of calcium and inorganic phosphate from the aqueous phase to the micellar phase. Depending on the physico-chemical conditions, these modifications of salt equilibria are more or less important and reversible.

## 8.2 Methods for analysing the mineral content in milk

To control the quality and the biochemical evolution during the manufacture of dairy products, it is useful to determine the total contents and the partitions of these minerals between the aqueous and micellar phases of milk. The analytical methods commonly used need, in a first step, adequate preparation of the sample before the determination of the mineral content.

### 8.2.1 Preparation of sample

*Ashing*

In some cases, proteins, fat and lactose interfere with the determination of the minerals contained in milk and it is useful to eliminate the organic matter. Dry or wet ashing are the possible ways to destroy this organic matter (Moreno-Torres *et al.*, 2000). Dry ash is the material that remains after removing the organic material by heating between 400 and 800ºC for several hours. Dry mineralisation led to the destruction of citrate and the transformation of organic phosphate (phosphate groups esterified to serine residues of caseins or to several small molecules such as pentose, hexoses, glycerol, serine and nucleotides) in inorganic phosphate. Moreover, if the dry ashing is performed at temperatures higher than 550ºC, chloride ions are lost by volatilisation. The dry ashes obtained can be used, after dissolution in dilute hydrochloric or nitric acid solution, for the determination of one or several specific ions. The dry ashing does not require reagents and it can be applied to relatively large amounts of sample. Wet ashing led to a destruction of organic matter by oxidation with various mixtures of nitric, sulfuric and perchloric acid. This preparation requires the use of pure acids and is limited by the amount of sample. A variant of wet ashing is the high pressure digestion technique in a sealed Teflon or glassy carbon vessel. The main advantage is a significantly reduced consumption of acid for digestion.

*Acid extraction*

Total mineral content can be obtained after an acid-extraction. Indeed, acidification of milk to a final pH of about 3.0 led to a total transfer of calcium, magnesium, inorganic phosphate and citrate associated with casein micelles toward the aqueous phase (Le Graët and Brulé, 1993). Frequently, acidification is performed using nitric, sulfuric, chlorhydric or trichloroacetic acid. In the last

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

case, all milk proteins and high molecular mass peptides are precipitated and the determination of minerals must be realised on the filtrate. It is noteworthy that during acid extraction, the organic phosphate present in the phosphoserine residues is never solubilised.

*Aqueous phase*

Dialysis, ultrafiltration, ultracentrifugation and rennet coagulation of milk can be used to recover specifically its aqueous phase (Davies and White, 1960; Sindhu and Roy, 1973a,b,c; Brulé *et al*., 1974; De la Fuente *et al.*, 1996). During the sample preparation by one of these techniques, it is imperative to work at controlled pH and temperature since salt equilibria are very dependent on these physico-chemical parameters. For dialysis and ultrafiltration, it is recommended to use a membrane with a molecular weight cut-off close to 10,000 Da to avoid the transfer of small molecules and proteins able to bind minerals in the dialysate or ultrafiltrate. For ultracentrifugation, typical conditions are 80,000*g* for 2 hours or 100,000*g* for 1 hour. For concentrations determined in the dialysate, rennet whey, ultrafiltrate or supernatant of ultracentrifugation to correspond to the real concentration in the milk, it is necessary to use a correction factor. Thus, a mineral concentration found in the aqueous phase must be multiplied by a 0.96 correcting factor which takes into account the excluded volume effect (Pierre and Brulé, 1981). Moreover, some slight differences in results can exist depending on the type of aqueous phase preparation. The calcium and magnesium concentrations found in the ultracentrifugal supernatants are generally more important than those determined in the ultrafiltrate. This difference is related to the presence of whey proteins and soluble caseins in the ultracentrifugal supernatant (and not in the ultrafiltrates).

IP Address: 129.132.208.100

*Micellar phase*

In some cases, it can be interesting to evaluate the concentrations of minerals associated with casein micelles. The preparation of this phase is possible by using ultracentrifugation, but the pellet obtained after ultracentrifugation, which corresponds to casein micelles, is not easy to resolubilise. To avoid these difficulties, different authors deduce the micellar contents by subtracting the concentration remaining in the aqueous phase from the total concentration.

### 8.2.2 Mineral quantifications

From samples prepared as described in Section 8.2.1, different techniques can be applied to determine the concentrations of the main ions present in milk. These techniques can be classified according to their principles. Molecular absorption spectrometry, complexometric methods combined with titration, electrochemical methods (ion selective electrode), atomic spectrometry, enzymatic methods and separative methods (chromatography or capillary electrophoresis) with different detection principles are the most used and described. Particular attention must be paid to the evaluation of the phosphorus content. Indeed, the

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

**Table 8.3** Different forms of phosphorus present in milk

| Phosphorus form | Chemical form | Location |
|---|---|---|
| Organic phosphorus (Po) | 1. Esterified to $\alpha s_1$-, $\alpha s_2$-, $\kappa$- and $\beta$-CN (phosphoseryl residues) | → Micellar phase |
| | 2. Esterified to small molecules (nucleotides, phosphorylated sugars) | → Aqueous phase |
| Inorganic phosphate (Pi) | 3. Associated to micellar calcium phosphate | → Micellar phase |
| | 4. Associated to calcium ($CaHPO_4$) | → Aqueous phase |
| | 5. Free ($H_2PO_4^-$/$HPO_4^{2-}$) | → Aqueous phase |

results of the analysis are very dependent on the sample preparation because phosphorus exits in the milk in different forms (Table 8.3). Moreover, mass concentrations can be expressed in different ways as element (P), phosphate ($PO_4$) and oxide ($P_2O_5$).

*Calcium, magnesium, sodium and potassium by atomic spectroscopic methods*
The methods most suitable for the rapid and accurate determination of the content of these cations in milk are atomic spectroscopic methods. Different techniques such as flame atomic absorption spectrometry, graphite furnace atomic absorption spectrometry, inductively coupled plasma atomic emission spectrometry and inductively coupled plasma mass spectrometry (Alkanani *et al*., 1994; Murcia *et al*., 1999; Chen and Jiang, 2002; Sola-Larranaga and Navarro-Blasco, 2009) are currently used but atomic absorption spectrometry remains the method of choice (Wenner, 1958; Murthy and Rhea, 1967; Brulé *et al*., 1974; IDF, 1987b; Powell Gaines and West, 1990; Pollman, 1991; Zucchetti and Contarini, 1993; Noël *et al*., 2008). In the phenomenon of atomic emission, thermally excited electrons returning to their 'ground states' (most stable configurations) emit quanta of light energy of wavelengths characteristic of each element. The complementary process, whereby atoms in the ground state absorb quanta and attain excited states, is known as atomic absorption. The amount of light absorbed at the characteristic wavelength increases with the number of atoms of the selected element in the light path.

*Calcium and magnesium by titration*
One classical method for the determination of calcium in milk involves the precipitation of calcium as calcium oxalate followed by titration of the oxalate with potassium permanganate solution (IDF, 1992a). Another method is its titration with the chelating agent ethylenediamine tetraacetate (EDTA), using as indicator a dye (murexide) that changes colour when it binds calcium. In a similar way, magnesium can be also determined by titration with EDTA. Magnesium concentration can be obtained as the difference between two titrations, one with an indicator like murexide that measures calcium and the other with

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

eriochrome black that detects both calcium and magnesium (Pearce, 1977; Chaplin, 1984).

*Calcium, sodium and chloride by selective electrodes*

Ion-selective electrodes are used in conjunction with a reference electrode to form a complete electrochemical cell. The measured potential differences (ion-selective electrode vs. outer reference electrode potentials) are linearly dependent on the logarithm of the activity of a given ion in solution. Several ion electrodes for measuring directly and selectively calcium, sodium and chloride in milk and dairy products have been reported and used successfully by different authors (Demott, 1968, 1988; Randell and Linklater, 1972; Geerts *et al*., 1983; Kindstedt *et al*., 1983; Allen and Neville, 1983; Kindstedt and Kosikowski, 1984; Silanikove *et al*., 2003; Lin *et al*., 2006).

*Calcium by spectrophotometric and fluorimeric methods*

Among the spectrophotometric methods for the determination of calcium, those reporting the use of *o*-cresolphthalein complexone as the chromogenic reagent and 2-amino-2-methylpropan-1-ol as a base solution are widely accepted (van Staden and van Rensburg, 1990; Herrero *et al*., 1992). The absorbance of the calcium–cresolphthalein complexone complex is measured at 580 nm and pH 12.0.

The calcium content of milk can also be evaluated in the presence of glyoxal bis(2-hydroxyanil), by forming a complex with calcium having an absorption maximum at 524 nm (Walstra and Jenness, 1984). More recently, Ekinci *et al.* (2005) report the determination of calcium concentrations in human milk with energy dispersive X-ray fluorescence. Gangidi and Metzger (2006) describe the determination of ionic calcium in skim milk with molecular probes (Fluo-5N and Rhod-5N) and front-face fluorescence spectroscopy. Afkhami *et al*. (2008) present a novel spectrophotometric method using ratio spectra–continuous wavelet transformation for the simultaneous determination of ternary mixtures of calcium, magnesium and zinc without prior separation steps. The methods are based on the complexation reaction of these elements with bromopyrogallo red at pH 9.4.

IP Address: 129.132.208.100

*Calcium by catalase enzyme electrode*

Akyilmaz and Kozgus (2009) have developed a new biosensor based on the activation of catalase enzyme by calcium ion. They determined calcium concentration in milk without pre-treatment.

*Chloride, inorganic phosphate and citrate by ionic chromatography and capillary electrophoresis*

The anions ($Cl^-$, $PO_4^{3-}$, $Cit^{3-}$) can be separated by anion-exchange chromatography. After elution and before detection by conductivity, the signal due to the eluant is suppressed. This suppression simplifies the detection of ions by maximising the signal to noise ratio and allows a high sensitivity analysis (Cox

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

*et al*., 1985; Gaucheron *et al*., 1996; Buldini *et al*., 2002). The determination of anions by capillary electrophoresis is also reported by several authors (Schmitt *et al*., 1993; Wu *et al.*, 1995; Saulnier *et al*., 1996; Rabiller-Baudry *et al*., 1998; Kuban *et al*., 1999; Braunschweig and Puhan, 1999; Izco *et al.*, 2003). As for ion chromatography, the separation principle is based on differences in the charge-to-mass ratio of the ions analysed.

*Phosphorus by spectrophotometric method*

In reducing conditions, phosphorus reacts with molybdate (or vanadate) to form phosphomolybdate which absorbs significantly in the visible spectral region (IDF, 1987a, 1990; Herrero *et al*., 1992; Reis Lima *et al*., 2003).

*Citrate by spectrophotometric methods*

The content of citrate can be evaluated by measuring absorbance of a condensation product of this compound with pyridine in acetic anhydride. The reaction is performed on a trichloroacetic acid filtrate of the milk (Marier and Boulet, 1958; White and Davies, 1963).

Another method for citrate determination is based upon the complexing properties of citrate for $Cu^{2+}$ ions. The detection is made by absorptiometry at 280 nm (Pierre and Brulé, 1983). In this method, it is necessary to work at a pH close to 5.0 and to have a protein-free sample. This Cu-complex method allows a good recovery and determination of diffusible citrate in milk. Moreover, sensitivity and accuracy are similar to those of reference methods and this method is easier and faster.

Citrate can also be determined by an enzymatic method involving the use of citrate lyase, malate dehydrogenase, and lactate dehydrogenase and $NADH,H^+$ (Mutzelburg, 1979; IDF, 1992b). After enzymatic reactions, the absorbance decrease of $NADH,H^+$, which is proportional to citric acid concentration, is determined at a wavelength of 340 nm.

*Chloride by spectrophotometric method and titration*

One of the most frequently used methods for the determination of chloride consists of the spectrometric measurement at 480 nm of the coloured iron(II) thiocyanate complex formed (Herrero *et al*., 1992). This anion can also be determined by titration with silver nitrate (Mohr method). Chloride meters are available commercially that automatically titrate chloride ions with silver ions generated internally (Herrington and Kleyn, 1960; Reis Lima *et al.*, 2003). When titration is complete, conductivity of the solution increases, which can be sensed by electrodes causing the titration to stop. The instrument uses the elapsed titration time to calculate the chloride content. Traditional methods for measuring sodium generally involve titration of chloride in the sample with silver nitrate with a colour indicator for the endpoint. These methods are specific for chloride and indirectly measure sodium ion.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

### 8.2.3 Theoretical calculation of the salt equilibria

The partition of ions between aqueous and micellar phases can be calculated theoretically. Today, these models of calculation constitute new and interesting tools to simulate salt equilibria in milk and dairy formulations containing different concentrations of minerals and caseins.

According to Holt (2004), calculations are performed from a generalised empirical formula for the nanoclusters containing calcium, magnesium, inorganic phosphate and citrate in association with a casein phosphorylated sequence named the phosphate centre. Moreover, in this model, the mole fractions of the individual caseins not complexed to the calcium phosphate through one or more of their phosphate centres are computed. Using this model, it is possible to calculate the partition of milk salts into diffusible and non-diffusible fractions. To validate the model, the author compared the calculated values and experimental values obtained by other authors and found a good correlation.

The model proposed by Mekmene *et al*. (2009) is an extension of that described by Holt *et al.* (1981). In this model, calculations are based on interactions between anions (phosphoseryl residues and carboxylic groups of casein molecules, inorganic phosphate, chloride and citrate) and cations (calcium, magnesium, potassium and sodium) according to their reciprocal affinity and considering pH, ionic strength, and solubility of calcium phosphate. The results obtained by these authors are also in good agreement with experimental data published in the literature.

IP Address: 129.132.208.100

## 8.3 Improving the mineral content in milk

### 8.3.1 Increase of the mineral content in milk

The mineral enrichment of milk, which is a common practice in the dairy industry, is realised for nutritional, functional or technological reasons. It is performed by addition of different salts or by concentration of milk by membrane technologies. In this section, particular attention is paid to the addition of calcium, sodium chloride, orthophosphate and calcium-chelatants.

*Addition of calcium*

Several hundred publications studying the effect of calcium enrichment of milk exist. There is an evident interest for the dairy industry to manufacture calcium-enriched dairy products, which could have better nutritional and technological properties than non-enriched milk. In this context of enrichment, the concentrations of calcium added are generally between 5 and 20 mM, i.e. 200 and 800 mg/l in calcium.

It is possible to add different types of salts of calcium which can be classified as a function of anions associated to calcium, solubility or origin of calcium compounds. Among these salts, gluconate, lactate, citrate, chloride, glycerol-phosphate, malate, oxalate, phosphate (tricalcium, dicalcium and monocalcium),

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

pyrophosphate, carbonate, sulfate, hydroxide and oxide are the most described (Fligner *et al*., 1988; Pirkul *et al*., 1997; Weaver, 1998; Udabage *et al*., 2000; Augustin and William, 2002; Philippe *et al*., 2003, 2004; Vyas and Tong, 2004). The most used calcium salts are calcium gluconate, calcium lactate, calcium citrate, calcium chloride, calcium carbonate and calcium phosphate. Citrate, carbonate and phosphate calcium salts are weakly soluble in milk and can sediment and produce undesirable organoleptic properties. Chloride, lactate and gluconate salts have good solubilities. In 1999, Ayling proposed the development of an ingredient composed of microcrystalline cellulose, carboxymethyl cellulose and calcium carbonate (Ayling, 1999). Today, another source of calcium more and more used by the dairy industry is the milk calcium phosphate (Harju, 2001). The composition of this salt depends on how it is recovered. It is composed of calcium and inorganic phosphate but it can contain lactose, whey proteins and other ions like chloride, sodium, and potassium.

If the enrichment is performed with insoluble calcium salts such as calcium phosphate, the salt equilibria are not modified (Philippe *et al*., 2004) and the physico-chemistry of milk is not affected. Only sediment is observed. The rate of sedimentation can be reduced if the size of calcium salts is lowered by physical treatment. On the other hand, if the calcium salts are soluble, the salt equilibria are modified. One part of the added calcium remains in the aqueous phase as the ionic form and the other part reacts with inorganic phosphate present in the aqueous phase. As this phase is saturated in calcium phosphate (Holt, 1997), phosphate and calcium concentrations decrease, suggesting a precipitation or association of this salt to the micellar phase (Tessier and Rose, 1958; Lin *et al*., 1972; Van Hooydonk *et al*., 1986; Gastaldi *et al*., 1994; Udabage *et al*., 2000; Philippe *et al*., 2003, 2004). At the same time, incorporation of soluble caseins into casein micelles and release of water from casein micelles occur. These changes in mineral, protein and water distributions induce a pH decrease (which can be kept constant by NaOH addition), increases in extrinsic fluorescence, turbidity and lightness of milks, and a decrease in zeta potential of casein micelles (Dalgleish, 1984; Munyua and Larsson-Raznikiewicz, 1980; Udabage *et al*., 2000; Philippe *et al*., 2003, 2004). Consequently to this micellar destabilisation, the characteristics of rennet coagulation are changed: the time of coagulation is reduced, the firmness of the gel increased and its ability to syneresis reduced. The cheese yield is also increased (Wolfschoon-Pombo, 1997). On the other hand, the enriched milks are in general less stable to heat treatment (Grandison, 1988; Augustin and Clarke, 1990; Jeurnink and de Kruif, 1995; Le Ray *et al*., 1998; Philippe *et al*. 2003; McKinnon *et al*., 2009).

All these results show that the enrichment of milk with an external source of calcium remains limited. With our present knowledge, a calcium-enriched milk containing more than 2 g/l of calcium with a good acceptability, i.e. without sedimentation and stable during and after heat treatment, is difficult to achieve. The reasons for this limitation in the enrichment are as follows:

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

- The low number of free phosphoseryl residues that are able to bind supplementary calcium (it is admitted that more than 90% of these residues are associated with calcium and magnesium in non-enriched milk)
- The supersaturation state in calcium phosphate of the aqueous phase (Holt, 1997).

In spite of these limitations, several authors propose different strategies by combining several approaches to reduce these effects. Thus, Guamis-Lopez and Quevedo-Terre (1996) have developed a mineral complex formed by the interaction of a soluble calcium salt and citrate. Fortification with this complex is possible without coagulation and sedimentation of milk. More recently, Williams *et al.* (2005) developed a strategy for the calcium fortification of liquid milk by adding a combination of orthophosphates with calcium chloride. In their case, they obtain a skim milk with a total calcium content between 2000 and 2300 mg/l. With the same objective, Singh *et al.* (2007) report the preparation of fortified milk with calcium salts. These milks were also heat-stabilised by addition of disodium phosphate.

*Addition of sodium chloride*

The industrial preparation of salted milk does not exist and the studies concerning the addition of this salt to milk are relatively limited. In some existing studies, the authors add to milk between 50 and 1000 mM NaCl. Under these conditions, the physico-chemical properties of milk are altered. A slight decrease in pH and a solubilisation of micellar calcium but not inorganic phosphate are described by several authors (Grufferty and Fox, 1985; Zoon *et al.*, 1989; Casiraghi and Lucisano, 1991; Le Graët and Brulé, 1993; Le Ray *et al*., 1998; Gaucheron *et al*., 2000; Huppertz and Fox, 2006). These results suggest exchanges of calcium linked directly to the phosphoseryl residues of the casein molecules with the added sodium. As a consequence of these exchanges, a weakening of the extent of binding strength between caseins is probable and increases in hydration of casein micelles and viscosity of milk are induced (Grufferty and Fox, 1985; Van Hooydonk *et al.*, 1986; Famelart *et al.*, 1996; Le Ray *et al*., 1998; Guillaume *et al*., 2002), although their size and the charge remain constant.

*Addition of orthophosphate*

Addition of orthophosphate, i.e. inorganic phosphate, to raw and differently processed milks (heated, concentrated and/or recombined milks) is performed in the dairy industry for different reasons. It can be added to improve heat stability (Sindhu, 1985; Augustin and Clarke, 1990; Pouliot and Boulet, 1991; Van Mil and De Koning, 1992; Montilla and Calvo, 1997; Le Ray *et al*., 1998) and stability after heat treatment (Harwalkar and Vreeman, 1978a,b). It can limit the formation of deposit in heat treatment equipment (Burdett, 1974; Joshi and Patel, 1986). Water transfer is increased by the presence of inorganic phosphate during drying of dairy proteins and reconstitution of dairy protein powders

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

(Schuck *et al*., 1999, 2002). On the other hand, addition of inorganic phosphate to milk is also performed to select milk to be submitted to UHT processing and to monitor the stability of UHT milk during storage (Ramsdell Test) (Ramsdell *et al*., 1931; Gaucher *et al.*, 2008, 2009).

In spite of these various applications, there are few studies describing and explaining the physico-chemical changes induced by addition of supplementary inorganic phosphate to milk. In the published studies, authors add it in a concentration range between 10 to 200 mM and it appears that the physico-chemical changes induced by these additions depend on the concentrations of added inorganic phosphate (Udabage *et al*., 2000; Gaucher *et al*., 2007). If the concentrations are less than 50 mM, inorganic phosphate can be considered as relatively inert. Experiments show that the major part of the added phosphate remains in the aqueous phase (Gaucher *et al*., 2007). In fact, calcium of the aqueous phase is preferentially combined with citrate rather than phosphate because the value of the association constant of calcium with $Cit^{3-}$ is $2.2 \times 10^5\,M^{-1}$ compared to $21\,M^{-1}$ for $H_2PO_4^-$ and $442\,M^{-1}$ for $HPO_4^{2-}$ (Mekmene *et al.*, 2009). For this reason, the combination of inorganic phosphate with calcium in the aqueous phase is probably low but significant. Indeed, one small part of the added inorganic phosphate can react with ionic calcium which is directly available in the aqueous phase. As the aqueous phase is saturated in calcium phosphate (Holt, 1997), decreases of calcium and inorganic phosphate concentrations in this phase occur, suggesting a precipitation or association of calcium phosphate in the micellar phase. These slight mineral changes do not modify strongly the physico-chemical properties of casein micelles. In the presence of higher concentrations of inorganic phosphate (more than 50 mM), the observed effects are different. The calcium concentration in the aqueous phase increases, indicating a displacement of this ion from the micellar to the aqueous phase. As the aqueous phase is saturated in calcium phosphate, the newly formed calcium phosphate becomes insoluble and precipitates again.

The results obtained by scanning electron microscopy and energy-dispersive X-ray spectroscopy for the phosphate-enriched milk confirmed the precipitation of brushite-type calcium phosphate salt (Gaucher *et al*., 2007). In parallel to these modifications of salt equilibria, changes in the organisation of casein micelles are observed. The decrease in lightness (which is related to particle concentration and structural organisation such as size and content in protein and minerals) and the increase of nitrogen content in the supernatant of ultra-centrifugation indicate a disruption of the casein micelle. All these modifications of micellar structure induce also changes in the interactions between water and casein molecules. These findings were in agreement with the increase in water content associated with the pellet obtained by ultracentrifugation of phosphate-enriched milk (Gaucher *et al*., 2007) and with increases in viscosity and gelation of milk in the presence of 100 mM phosphate (Fox *et al.*, 1965). This interpretation is also in accordance with results obtained by Schuck *et al.* (1999) indicating that the addition of phosphate increases water transfer during drying of dairy concentrates and reconstitution of powders.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

*Addition of calcium-chelatants*

The chelating agents most used are citrate, EDTA, oxalate, pyrophosphate and polyphosphate. The mechanism of action of these chelating compounds is relatively well described. They have high affinities for calcium and are able to react with it to form salts. Consequently to this salt formation, the mineral equilibria are altered and release of calcium and inorganic phosphate from the casein micelles to the aqueous phase is observed (Odagiri and Nickerson, 1964, 1965; Morr, 1967; Brulé and Fauquant, 1981; Casiraghi and Lucisano, 1991; Gatti *et al.*, 1999). However, the type of calcium solubilised (calcium bound directly to phosphoseryl residues and/or calcium bound to inorganic phosphate) is not well defined. To have an idea, it is necessary to take into account the different associations between calcium and different counter ions. The association constants between calcium–organic phosphate, calcium–citrate$^{-}$ and calcium–$HPO_4$ are $2.2 \times 10^4$, $2.2 \times 10^5$ and 442 $M^{-1}$, respectively (Mekmene *et al.*, 2009). Firstly, the comparison of these values indicates that citrate has the highest affinity towards calcium compared to both types of phosphate. Secondly, calcium is more strongly bound to phosphoseryl residues than to inorganic phosphate. These comparisons suggest that citrate solubilises preferentially the calcium bound to inorganic phosphate compared to the calcium bound to phosphoseryl residues. Even if the type of calcium solubilised is not precisely known, the micellar calcium phosphate is solubilised and casein micelles are disrupted (Odagiri and Nickerson, 1964; Morr, 1967; Rollema and Brinkhuis, 1989; Johnston and Murphy, 1992; McCrae and Muir, 1995; Le Ray *et al.*, 1998).

Thanks to these properties of calcium chelation, some of these chelatants are used in the dairy industry. Their additions improve the heat stability and storage life of different milks (Sweetsur and Muir, 1980; Boumpa *et al.*, 2008; Faka *et al.*, 2009) and prevent deposit formation on heat exchanger and membrane surfaces.

IP Address: 129.132.208.100

*Use of membrane technologies*

Another way to increase the calcium and phosphate contents is to concentrate the casein micelles because they contain in their structure the micellar calcium phosphate. This concentration can be realised by microfiltration, ultrafiltration or nanofiltration. Using microfiltration and ultrafiltration, the increase in calcium concentration in the retentate is directly related to the concentration of casein micelles:

$$[Ca]_{retentate} = [Ca]_{milk} + ([Ca]_{milk} - [Ca]_{permeate})(\text{concentration factor} - 1)$$

If the $[Ca]_{milk}$ and $[Ca]_{permeate}$ are 30 and 10 mM, respectively, the [Ca] in the retentate concentrated 1.5 and 2 times would be 40 and 50 mM. During concentration, the contents of casein and phosphate (organic and inorganic) increase in the same proportion, whereas the mineral content in the aqueous phase is not modified, since these membrane processes are essentially an equilibrium dialysis. Using nanofiltration, the totality of the calcium (present in the micellar

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

and aqueous phases) is retained in the retentate and its increase is directly proportional to the concentration factor.

### 8.3.2 Reduction of the mineral content in milk

*Use of membrane technologies*

During the protein concentration by microfiltration, ultrafiltration or nanofiltration, a diafiltration can be performed. This step, which consists of dilution of the retentate (in general with 4–5 volumes of water), allows the elimination of lactose, hydrosoluble vitamins, small molecules containing nitrogen, whey proteins (only for the microfiltration) and minerals present in the aqueous phase, i.e. calcium, magnesium, sodium and potassium for cations and inorganic phosphate, chloride and citrate for anions. At the end of the filtration, the retentate containing proteins and minerals associated (micellar calcium phosphate) can be spray dried. After reconstitution of the powder in water, the casein micelles have an aqueous phase containing a very low concentration of minerals. A typical example is the preparation of a product called native phosphocaseinate (Fauquant *et al*., 1988; Pierre *et al*., 1992; Schuck *et al*., 1994a,b).

To increase the demineralisation of casein micelles, it is also possible to apply microfiltration or ultrafiltration on milk previously acidified or containing chelatants. In both cases, calcium and inorganic phosphate are transferred in the aqueous phase and eliminated in the permeate. The level of demineralisation will depend on the acidification pH and the concentration of added chelatants.

A specific reduction of the mineral content can also be obtained by using nanofiltration (Jeantet, 1995; Kiyoshi *et al*., 1999). These authors showed that the protein, lactose, calcium, magnesium, and inorganic phosphate are retained at more than 95% whereas the chloride, sodium and potassium are in the nanofiltrate. The factor of concentration determines the level of the demineralisation in monovalent ions. The nanofiltered milk (which is rich in calcium and lactose and depleted in sodium, potassium and chloride) can be transformed in various dairy products, which are different from those obtained with 'normal' milk. This milk is interesting for the development of specific dairy products depleted in sodium or with a particular taste (Matsui *et al*., 2006). Mucchetti *et al.* (2000) showed that Quarg obtained using nanofiltration retentate is naturally sweeter than traditional fresh cheese and has a high calcium content and no bitter taste.

*Use of ion-exchange chromatographies*

The removal of one part of ions present in milk is possible by using a combination of cation and anion exchangers. In the presence of ions, the resins $-H^+$ or $-NH_4^+$ and $-OH^-$ or $-HCO_3^-$ bind $Ca^{2+}$, $Mg^{2+}$, $Na^+$, $K^+$, $Cl^-$, citrate and phosphate ions and release $H^+$ or $NH_4^+$ and $OH^-$ or $HCO_3^-$ ions. This operation can be used for the manufacture of milk depleted in sodium or calcium. Thus, Nakazawa and Hosono (1989) prepared, from milk containing 548, 1529, 126 and 1135 mg/kg sodium, potassium, magnesium and calcium ions, respectively,

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

modified milk containing after chromatography 65, 2348, 120 and 1200 mg/kg, respectively, of these same ions. Ranjith *et al*. (1999) used this process in 'batch' to produce milk with reduced contents in calcium and magnesium. The levels of reduction in calcium depend on the resin used and the time of contact and vary between 10 and 80%. The main consequences of these demineralisations are (1) a decrease of the phosphorus concentration; (2) increases of sodium and/or potassium concentrations, which are the counterions of the resins used; and (3) a significant decrease of the whiteness of milk. The mechanisms of the demineralisation of casein micelles are close to those observed after addition of chelating agents described previously.

## 8.4 Conclusions

The mineral fraction of milk has been subjected to extensive investigations for over 60 years. Even if some questions remain on the exact nature, organisation and role of the micellar calcium phosphate in the structure and stability of casein micelles, we have a good knowledge of the composition and distribution of this fraction in milk, which has been made possible by performing sample preparation and analytical methods to separate and quantify the different minerals of milk. In parallel and in a context of increase of the nutritional, functional or technological properties of milk, a lot of research has focused on the improvement of milk by modifying its mineral content. Technically, it is possible to add or eliminate minerals in the milk. However, it is admitted that these enrichments or depletions are not totally without effect on the milk system. Depending on the type of mineral added or depleted, the physico-chemical properties of milk are more or less modified, especially the salt equilibria, which have an impact on the micellar stability.

IP Address: 129.132.208.100

## 8.5 References

AFKHAMI A, MADRAKIAN T, ABBASI-TARIGHAT M (2008), 'Simultaneous determination of calcium, magnesium and zinc in different foodstuffs and pharmaceutical samples with continuous wavelet transforms', *Food Chem*, 109, 660–669.

AKYILMAZ E, KOZGUS O (2009), 'Determination of calcium in milk and water samples by using catalase enzyme electrode', *Food Chem*, 115, 347–351.

ALKANANI T, FRIEL J K, JAKSON S E, LONGERICH H P (1994), 'Comparison between digestion procedures for the multielemental analysis of milk by inductively coupled plasma mass spectrometry', *J Agric Food Chem*, 42, 1965–1970.

ALLEN J C, NEVILLE M C (1983), 'Ionized calcium in human milk determined with a calcium-selective electrode', *Clin Chem*, 29, 858–861.

AUGUSTIN M A, CLARKE P T (1990), 'Effects of added salts on the heat stability of recombined concentrated milk', *J Dairy Res*, 57, 213–226.

AUGUSTIN M A, WILLIAM R P W (2002), 'Technological aspects of calcium fortification of milk and dairy products', *Food Austr*, 54, 131–133.

© Woodhead Publishing Limited, 2010

Downloaded From Vet-eBooks.com

AYLING G (1999), 'Calcium fortified milk in Asia using co-processed microcrystalline cellulose', *Third International Symposium on Recombined Milk and Milk Products*, Penang, 23–26 May, 117–118.

BOUMPA T, TSIOULPAS A, GRANDISON A S, LEWIS M J (2008), 'Effects of phosphates and citrate on sediment formation in UHT goats' milk', *J Dairy Res*, 75, 160–166.

BRAUNSCHWEIG M, PUHAN Z (1999), 'Correlation between κ-casein variants and citrate content in milk quantified by capillary electrophoresis', *Int Dairy J*, 9, 709–713.

BRULÉ G, FAUQUANT J (1981), 'Mineral balance in skim-milk and milk retentate: effect of physicochemical characteristics of the aqueous phase', *J Dairy Res*, 48, 91–97.

BRULÉ G, MAUBOIS J L, FAUQUANT J (1974), 'Etude de la teneur en éléments minéraux des produits obtenus lors de l'ultrafiltration de lait sur membrane', *Lait*, 54, 600–615.

BULDINI P L, CAVALLI S, SHARMA J L (2002), 'Matrix removal for the ion chromatographic determination of some trace elements in milk', *Microchem J*, 72, 277–284.

BURDETT M (1974), 'The effect of phosphates in lowering the amount of deposit formation during the heat treatment of milk', *J Dairy Res*, 41, 123–129.

CASIRAGHI E, LUCISANO M (1991), 'Rennet coagulation of milk retentates. Effect of the addition of sodium chloride and citrate before ultrafiltration', *Milchwissenschaft*, 46, 775–778.

CHAPLIN L C (1984), 'Complexometric titration of milk for calcium plus magnesium, and calcium only', *J Dairy Res*, 51, 477–480.

CHEN K L, JIANG S J (2002), 'Determination of calcium, iron and zinc in milk powder by reaction cell inductively coupled plasma mass spectrometry', *Anal Chim Acta*, 470, 223–228.

COX D, HARRISON G, JANDIK P, JONES W (1985), 'Application of ion chromatography in the food and beverage industry', *Food Techn*, 7, 41–44.

DALGLEISH D G (1984), 'Measurement of electrophoretic mobilities and zeta-potentials of particles from milk using laser doppler electrophoresis', *J Dairy Res*, 51, 425–438.

DAVIES D T, WHITE J C D (1960), 'The use of ultrafiltration and dialysis in isolating the aqueous phase of milk and in determining the partition of milk constituents between the aqueous and disperse phases', *J Dairy Res*, 27, 171–190.

DE LA FUENTE M A (1998), 'Changes in the mineral balance of milk submitted to technological treatments', *Trends Food Sci Techn*, 9, 417–428.

DE LA FUENTE M A, FONTECHA J, JUAREZ M (1996), 'Partition of main and trace minerals in milk: effect of ultracentrifugation, rennet coagulation and dialysis on soluble phase separation', *J Agric Food Chem*, 44, 1988–1992.

DEMOTT B J (1968), 'Ionic calcium in milk and whey', *J Dairy Sci*, 51, 1008–1012.

DEMOTT B J (1988), 'Determination of calcium in cottage cheese by titration', *J Food Protect*, 51, 802–803.

EKINCI N, EKINCI R, POLAT R, BUDAK G (2005), 'The determination of calcium concentrations in human milk with energy dispersive X-ray fluorescence', *J Quantit Spectr Radiat Transf*, 91, 155–160.

FAKA M, LEWIS M J, GRANDISON A S, DEETH H (2009), 'The effect of free $Ca^{2+}$ on the heat stability and other charcteristics of low-heat skim milk powder', *Int Dairy J*, doi: 10.1016/j.idairyj.2008.12.006

FAMELART M H, LEPESANT F, GAUCHERON F, LE GRAËT Y, SCHUCK P (1996), 'pH-Induced physicochemical modifications of native phosphocaseinate suspensions: influence of aqueous phase', *Lait*, 76, 445–460.

FAUQUANT J, MAUBOIS J L, PIERRE A (1988), 'Microfiltration du lait sur membrane minérale', *Tech Lait*, 1028, 21–23.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-eBooks.com

FLIGNER K, LINDAMOOD J B, HANSEN P M T (1988), 'Fortification of low-fat plain yogurt with calcium gluconate', *Cult Dairy Prod J*, 23, 5–9.

FOX K K, HARPER M K, HOLSINGER V H, PALLANSCH M J (1965), 'Gelation of milk solids by orthophosphate', *J Dairy Sci*, 48, 179–185.

GANGIDI R R, METZGER L E (2006), 'Ionic calcium determination in skim milk with molecular probes and front-face fluorescence spectroscopy: simple linear regression', *J Dairy Sci*, 89, 4105–4113.

GASTALDI E, PELLEGRINI O, LAGAUDE A, TARODO DE LA FUENTE B (1994), 'Functions of added calcium in acid milk coagulation', *J Food Sci*, 59, 310–312.

GATTI C A, ALVAREZ E M, SUAREZ SALA V (1999), 'Effect of the anion citrate on the mineral composition of artificial casein micelles', *J Agric Food Chem*, 47, 141–144.

GAUCHER I, PIOT M, BEAUCHER E, GAUCHERON F (2007), 'Physico-chemical characterization of phosphate-added skim milk', *Int Dairy J*, 17, 1375–1383.

GAUCHER I, MOLLÉ D, GAGNAIRE V, GAUCHERON F (2008), 'Effects of storage temperature on physico-chemical characteristics of semi-skimmed UHT milk', *Food Hydrocolloids*, 22, 130–143.

GAUCHER I, MOLLÉ D, GAGNAIRE V, LÉONIL J, ROUSSEAU F, GAUCHERON F (2009), 'Destabilization of commercial UHT milks: proteolysis and changes in milk particles', *Milchwissenschaft*, 64, 43–47.

GAUCHERON F (2004), *Minéraux et Produits Laitiers*, Paris, Tec & Doc.

GAUCHERON F, LE GRAËT Y, PIOT M, BOYAVAL E (1996), 'Determination of anions of milk by ion chromatography', *Lait*, 76, 433–443.

GAUCHERON F, LE GRAËT Y, BRIARD V (2000), 'Effect of NaCl addition on the mineral equilibrium of concentrated and acidified casein micelles', *Milchwissenschaft*, 55, 82–86.

GEERTS J P, BEKHOF J J, SCHERJON J W (1983), 'Determination of calcium ion activities in milk with an ion-selective electrode. A linear relationship between the logarithm of time and the recovery of the calcium ion activity after heat treatment', *Neth Milk Dairy J*, 37, 197–211.

GRANDISON A S (1988), 'Effects of natural (or seasonal) variation in concentration of components of milk and addition of divalent cations on UHT processing characteristics', *J Soc Dairy Technol*, 41, 117–119.

GRUFFERTY M B, FOX P F (1985), 'Effect of added NaCl on some physicochemical properties of milk', *Ir J Food Sci Technol*, 9, 1–9.

GUAMIS-LOPEZ B, QUEVEDO-TERRE J M (1996), 'Calcium enrichment of skimmed milk given UHT treatment', *Alimentaria*, 271, 79–82.

GUILLAUME C, MARCHESSEAU S, LAGAUDE A, CUQ J L (2002), 'Effect of salt addition on the micellar composition of milk subjected to pH reversible $CO_2$ acidification', *J Dairy Sci*, 85, 2098–2105.

HARJU M (2001), 'Milk sugar and minerals as ingredients', *Int J Dairy Techn*, 54, 61–63.

HARWALKAR V R, VREEMAN H J (1978a), 'Effect of added phosphates and storage on changes in ultra-high temperature short-time sterilized concentrated skim-milk. 1. Viscosity, gelation, alcohol stability, chemical and electrophoretic analysis of proteins', *Neth Milk Dairy J*, 32, 94–111.

HARWALKAR V R, VREEMAN H J (1978b), 'Effect of added phosphates and storage on changes in ultra-high temperature short-time sterilized concentrated skim-milk. 2. Micelle structure', *Neth Milk Dairy J*, 32, 204–216.

HERRERO M A, ATIENZA J, MAQUIEIRA A, PUCHADES R (1992), 'Flow injection

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

spectrophotometric determination of calcium, phosphate and chloride ions in milk', *Analyst*, 117, 1019–1024.

HERRINGTON B L, KLEYN D H (1960), 'A potentiometric method for the determination of chloride in milk', *J Dairy Sci*, 43, 1050–1057.

HOLT C (1985), 'The milk salts: their secretion, concentration and physical chemistry', in Fox P F, *Developments in Dairy Chemistry, Vol. 3: Lactose and Minor Constituents*, London, Elsevier, 143–181.

HOLT C (1997), 'The milk salts and their interaction with casein' in Fox P F, *Developments in Dairy Chemistry, Vol. 3: Lactose, Water, Salts and Vitamins*, London, Chapman & Hall, 233–254.

HOLT C (2004), 'An equilibrium thermodynamic model of the sequestration of calcium phosphate by casein micelles and its application to the calculation of the partition of salts in milk', *Eur Biophys*, 33, 421–434.

HOLT C, HORNE D (1996), 'The hairy casein micelle: evolution of the concept and its implication for dairy technology', *Neth Milk Dairy J*, 50, 85–111.

HOLT C, DALGLEISH D G, JENNESS R (1981), 'Calculation of the ion equilibria in milk diffusate and comparison with experiment', *Anal Biochem*, 113, 154–163.

HUPPERTZ T, FOX P F (2006), 'Effect of NaCl on some physico-chemical properties of concentrated bovine milk', *Int Dairy J*, 16, 1142–1148.

INTERNATIONAL DAIRY FEDERATION (1987a), 'Fromages et fromages fondus, détermination de la teneur en phosphore total, méthode photométrique', Norme 33C.

INTERNATIONAL DAIRY FEDERATION (1987b), 'Lait sec, détermination des teneurs en sodium et potassium, méthode par spectrométrie d'émission de flamme', Norme 119A.

INTERNATIONAL DAIRY FEDERATION (1990), 'Lait, détermination de la teneur en phosphore total (méthode spectrométrique)', Norme 42B.

INTERNATIONAL DAIRY FEDERATION (1992a), 'Lait, détermination de la teneur en calcium, méthode titrimétrique', Norme 36A.

INTERNATIONAL DAIRY FEDERATION (1992b), 'Fromages et fromages fondus, détermination de la teneur en acide citrique, méthode enzymatique', Norme 34 C.

IZCO J M, TORMO M, HARRIS A, TONG P S, JIMENEZ-FLORES R (2003), 'Optimization and validation of a rapid method to determine citrate and inorganic phosphate in milk by capillary electrophoresis', *J Dairy Sci*, 86, 86–95.

JEANTET R (1995), 'Nanofiltration de liquides laitiers', Thèse de doctorat, Ecole Nationale Supérieure Agronomique de Rennes, France.

JEURNINK T J M, DE KRUIF K G (1995), 'Calcium concentration in milk in relation to heat stability and fouling', *Neth Milk Dairy J*, 49, 151–165.

JOHNSTON D E, MURPHY R J (1992), 'Effects of some calcium-chelating agents on the physical properties of acid-set milk gels', *J Dairy Res*, 59, 197–208.

JOSHI R M, PATEL A A (1986), 'Heat-induced deposit formation and its relationship to heat stability in a blend of recombined and fresh milk', *J Dairy Res*, 53, 323–328.

KINDSTEDT P S, KOSIKOWSKI F V (1984), 'Measurement of sodium chloride in cheese by a simple sodium ion electrode method', *J Dairy Sci*, 67, 879–883.

KINDSTEDT P S, MATTICK L R, KOSIKOWSKI F V (1983), 'Simple selective sodium ion electrode measure of sodium in cheese', *J Dairy Sci*, 66, 988–993.

KIYOSHI K, KAZUHIRO H, TOYOFUMI M (1999), 'Production of low sodium milk by nanofiltration membrane process', *Sci Ind*, 73, 108–113.

KUBAN P, OLDHOFF O, KARLBERG B (1999), 'Direct determination of small cations in

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

proteinaceous samples using a flow injection–capillary electrophoresis system', *J Chromatogr A*, 857, 321–326.

LE GRAËT Y, BRULÉ G (1993), 'Equilibres minéraux du lait: influence du pH et de la force ionique', *Lait*, 73, 51–60.

LE RAY C, MAUBOIS J L, GAUCHERON F, BRULÉ G, PRONNIER P, GARNIER F (1998), 'Heat stability of reconstituted casein micelle dispersions: changes induced by salt addition', *Lait*, 78, 375–390.

LIN M, LEWIS M J, GRANDISON A S (2006), 'Measurement of ionic calcium in milk', *Int J Dairy Techn*, 59, 192–199.

LIN S H C, LEONG S L, DEWAN R K, BLOOMFIELD V A, MORR C V (1972), 'Effect of calcium ion on the structure of native bovine casein micelles', *Biochemistry*, 11, 1818–1821.

LYSTER R L J, MANN S, PARKER S B, WILLIAMS R J P (1984), 'Nature of micellar calcium phosphate in cow's milk as studied by high resolution electron microscopy', *Biochim Biophys Acta*, 801, 315–317.

MARCHIN S, PUTAUX J L, PIGNON F, LÉONIL J (2007), 'Effects of the environmental factors on the casein micelle structure studied by cryo-transmission electron microscopy and small-angle X-ray scattering/ultrasmall-angle X-ray scattering', *J Chem Phys*, 126, 045101.

MARIER J R, BOULET M (1958), 'Direct determination of citric acid in milk with an improved pyridine–acetic anhydride method', *J Dairy Sci*, 41, 1683–1692.

MATSUI H, MIZOTA Y, SUMI M, IKEDA M, IWATSUKI K (2006), 'Sensory characteristics of membrane treated-milk (Studies on the sensory characteristics and physicochemical properties of UHT processed milk)', *J Japanese Soc Food Sci Technol*, 53, 644–650.

MCCRAE C H, MUIR D D (1995), 'Heat stability of milk', in Fox P F, *Heat-induced Changes in Milk*, International Dairy Federation, Brussels, 206–230.

MCGANN T C A, BUCHHEIM W, KEARNEY R D, RICHARDSON T (1983), 'Composition and ultrastructure of calcium phosphate–citrate complexes in bovine milk systems', *Biochim Biophys Acta*, 760, 415–420.

MCKINNON I R, YAP S E, AUGUSTIN M A, HEMAR Y (2009), 'Diffusing-wave spectroscopy investigation of heated reconstituted skim milks containing calcium chloride', *Food Hydrocolloids*, 23, 1127–1133.

MEKMENE O, LE GRAËT Y, GAUCHERON F (2009), 'A model for predicting salt equilibria in milk and mineral-enriched milks', *Food Chem*, 116, 233–239.

MONTILLA A, CALVO M M (1997), 'Goat's milk stability during heat treatment: effect of pH and phosphates', *J Agric Food Chem*, 45, 931–934.

MORENO-TORRES R, NAVARRO M, RUIZ-LOPEZ M D, ARTACHO R, LOPEZ C (2000), 'Comparison of wet and dry mineralisation procedures for determining calcium and phosphorus in cow's milk', *Australian J Dairy Techn*, 55, 23–27.

MORR C V (1967), 'Some effects of pyrophosphate and citrate ions upon the colloidal caseinate–phosphate micelles and ultrafiltrate of raw and heated skimmilk', *J Dairy Sci*, 50, 1038–1044.

MUCCHETTI G, ZARDI G, ORLANDINI F, GOSTOLI C (2000), 'The pre-concentration of milk by nanofiltration in the production of Quarg-type fresh cheeses', *Lait*, 80, 43–50.

MUNYUA J K, LARSSON-RAZNIKIEWICZ M (1980), 'The influence of $Ca^{2+}$ on the size and light scattering properties of casein micelles', *Milchwissenschaft*, 35, 748–749.

MURCIA M A, VERA A, MARTINEZ-TOMÉ M, MUNOZ A, HERNANDEZ-CORDOBA M, ORTIZ-GONZALEZ R (1999), 'Fast determination of the Ca, Mg, K, Na and Zn contents in milk and nondairy imitation milk using ICP-AES without mineralization stage',

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

*Lebensm-Wiss u-Technol*, 32, 175–179.

MURTHY G K, RHEA U (1967), 'Determination of major cations in milk by atomic absorption spectrometry', *J Dairy Sci*, 50**,** 313–317.

MUTZELBURG I D (1979), 'An enzymatic method for the determination of citrate in milk', *Australian J Dairy Techn*, 34, 82–84.

NAKAZAWA Y, HOSONO A (1989), 'Manufacture of low sodium milk by ion exchange', *Milchwissenschaft*, 44, 29–31.

NELSON L S, HOLT C, HUKINS D W L (1989), 'The EXAFS spectra of poorly crystalline calcium phosphate preparations from heated milk', *Physica B*, 158, 103–104.

NOËL L, CARL M, VASTEL C, GUÉRIN T (2008), 'Determination of sodium, potassium, calcium and magnesium content in milk products by flame atomic absorption spectrometry (FAAS): A joint ISO/IDF collaborative study', *Int Dairy J*, 18, 899–904.

ODAGIRI S, NICKERSON T A (1964), 'Complexing of calcium by hexametaphosphate, oxalate, citrate, and EDTA in milk. I. Effects of complexing agents on turbidity and rennet coagulation', *J Dairy Sci*, 47, 1306–1309.

ODAGIRI S, NICKERSON T A (1965), 'Complexing of calcium by hexametaphosphate, oxalate, citrate, and ethylenediamine-tetraacetate in milk. II. Dialysis of milk containing complexing agents', *J Dairy Sci*, 48, 19–22.

PEARCE K N (1977), 'The complexometric determination of calcium in dairy products', *New Zealand J Dairy Sci Techn*, 12, 113–115.

PHILIPPE M, GAUCHERON F, LE GRAËT Y, MICHEL F, GAREM A (2003), 'Physicochemical characterization of calcium-supplemented skim milk', *Lait*, 83, 45–59.

PHILIPPE M, GAUCHERON F, LE GRAËT Y (2004), 'Physicochemical characteristics of calcium supplemented skim milk: comparison of three soluble calcium salts', *Milchwissenschaft*, 59, 498–504.

PIERRE A, BRULÉ G (1981), 'Mineral and protein equilibria between the colloidal and soluble phases of milk at low temperature', *J Dairy Res*, 48, 417–428.

PIERRE A, BRULÉ G (1983), 'Dosage rapide du citrate dans l'ultrafiltrat de lait par complexation cuivrique', *Lait*, 63, 66–74.

PIERRE A, FAUQUANT J, LE GRAËT Y, PIOT M, MAUBOIS J L (1992), 'Préparation de phosphocaséinate natif par microfiltration sur membrane', *Lait*, 72, 461–474.

PIRKUL T, TEMIZ A, ERDEM Y K (1997), 'Fortification of yoghurt with calcium salts and its effect on starter microorganisms and yoghurt quality', *Int Dairy J*, 7, 547–552.

POLLMAN R M (1991), 'Atomic absorption spectrophotometric determination of calcium and magnesium and colorimetric determination of phosphorus in cheese: collaborative study', *J Assoc Off Anal Chem*, 74, 27–31.

POULIOT Y, BOULET M (1991), 'Seasonal variations in the heat stability of concentrated milk: effect of added phosphate and pH adjustment', *J Dairy Sci*, 74, 1157–1162.

POWELL GAINES T, WEST J W (1990), 'Determination of calcium and phosphorus in milk', *J Sci Food Agric*, 51, 207–213.

RABILLER-BAUDRY M, CHAUFER B, JEANTET R (1998), 'Optimisation of the background electrolyte for the determination of anions by capillary electrophoresis: application to sweet whey', *Lait*, 78, 333–339.

RAMSDELL G A, JOHNSON W M T, EVANS F R (1931), 'A test for the detection of milk unstable to heat', *J Dairy Sci*, 14, 93–106.

RANDELL A W, LINKLATER P M (1972), 'The rapid analysis of cheddar cheese. I. The determination of salt content using an electrode specific for chloride ion', *Australian J Dairy Techn*, 27, 51–53.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

RANJITH H M P, LEWIS M J, MAW D (1999), 'Production of calcium-reduced milks using an ion-exchange resin', *J Dairy Res*, 66, 139–144.

REIS LIMA M J, FERNANDES S M V, RANGEL A O S S (2003), 'Sequential injection titration of chloride in milk with potentiometric detection', *Food Cont*, 15, 609–613.

ROLLEMA H S, BRINKHUIS J A (1989), 'A $^{1}$H-NMR study of bovine casein micelles; influence of pH, temperature and calcium ions on micellar structure', *J Dairy Res*, 56, 417–428.

SAULNIER F, CALCO M, HUMBERT G, LINDEN G (1996), 'Composition minérale et organique de différents lactosérums acides industriels, analysée par électrophorèse capillaire', *Lait*, 76, 423–432.

SCHMIDT D G (1982), 'Association of caseins and casein micelle structure', in Fox P F, *Developments in Dairy Chemistry*, London, Elsevier, 61–86.

SCHMITT M, SAULNIER F, MALHAUTIER L, LINDEN G (1993), 'Effect of temperature on the salt balance of milk studied by capillary ion electrophoresis', *J Chromatogr*, 640, 419–424.

SCHUCK P, PIOT M, MÉJEAN S, FAUQUANT J, BRULÉ G, MAUBOIS J L (1994a), 'Déshydratation des laits enrichis en caséine micellaire par microfiltration; comparaison des propriétés des poudres obtenues avec celles d'une poudre de lait ultra-propre', *Lait*, 74, 47–63.

SCHUCK P, PIOT M, MÉJEAN S, LE GRAËT Y, FAUQUANT J, BRULÉ G, MAUBOIS J L (1994b), 'Déshydratation par atomisation de phosphocaseinate natif obtenu par microfiltration sur membrane', *Lait*, 74, 375–388.

SCHUCK P, BRIARD V, MÉJEAN S, PIOT M, FAMELART M H, MAUBOIS J L (1999), 'Dehydration by desorption and by spray drying of dairy proteins: influence of the mineral environment', *Drying Technol*, 17, 1347–1357.

SCHUCK P, DAVENEL P, MARIETTE F, BRIARD V, MÉJEAN S, PIOT M (2002), 'Rehydration of casein powders: effects of added mineral salts and salt addition methods on water transfer', *Int Dairy J*, 12, 51–57.

SILANIKOVE N, SHAPIRO F, SHAMAY A (2003), Use of an ion-selective electrode to determine free Ca ion concentration in the milk of various mammals', *J Dairy Res*, 70, 241–243.

SINDHU J S (1985), 'Influence of sodium phosphate on the heat stability of buffalo milk and its concentrate', *J Food Proc Pres*, 9, 57–64.

SINDHU J S, ROY N K (1973a), 'Partitioning of buffalo milk minerals. 1. Study through dialysis', *Milchwissenschaft*, 28, 573–575.

SINDHU J S, ROY N K (1973b), 'Partitioning of buffalo milk minerals. 2. Study through ultracentrifugation', *Milchwissenschaft*, 31, 479–483.

SINDHU J S, ROY N K (1973c), 'Partitioning of buffalo milk minerals. 3. Study through rennet coagulation', *Milchwissenschaft*, 31, 671–673.

SINGH G, ARORA S, SHARMA G S, SINDHU J S, KANSAL V K, SANGWAN R B (2007), 'Heat stability and calcium bioavailability of calcium-fortified milk', *LWT Food Science and Technology*, 40, 625–631.

SOLA-LARRANAGA C, NAVARRO-BLASCO I (2009), 'Optimization of a slurry dispersion method for minerals and trace elments analysis in infant formulae by ICP OES and FAAS', *Food Chem*, 115, 1048–1055.

SWEETSUR A W M, MUIR D D (1980), 'The use of permitted additives and heat-treatment to optimize the heat-stability of skim milk and concentrated skim milk', *J Soc Dairy Technol*, 33, 101–105.

TESSIER H, ROSE D (1958), 'Calcium ion concentration in milk', *J Dairy Sci*, 41, 351–359.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded From Vet-eBooks.com

UDABAGE P, MCKINNON I R, AUGUSTIN M A (2000), 'Mineral and casein equilibria in milk: effects of added salts and calcium-chelating agents', *J Dairy Res*, 67, 361–370.

VAN HOOYDONK A C M, HAGEDOORN H G, BOERRIGTER I J (1986), 'The effect of various cations on the renneting of milk', *Neth Milk Dairy J*, 40, 369–390.

VAN MIL P J J M, DE KONING J (1992), 'Effect of heat treatment, stabilizing salts and seasonal variation on heat stability of reconstituted concentrated skim milk', *Neth Milk Dairy J*, 46, 169–182.

VAN STADEN J F, VAN RENSBURG A (1990), 'Simultaneous determination of total and free calcium in milk by flow injection', *Analyst*, 115, 605–608.

VYAS H K, TONG P S (2004), 'Impact of source and level of calcium fortification on the heat stability of reconstituted skim milk powder', *J Dairy Sci*, 87, 1177–1180.

WALSTRA P, JENNESS R (1984), *Dairy Chemistry and Physics*, New York, Wiley Interscience.

WEAVER C M (1998), 'Calcium in food fortification strategies', *Int Dairy J*, 8, 443–449.

WENNER V R (1958), 'Rapid determination of milk salts and ions. I. Determination of sodium, potassium, magnesium and calcium by flame spectrophotometry', *J Dairy Sci*, 41, 761–768.

WHITE J C D, DAVIES D T (1963), 'The determination of citric acid in milk and milk sera', *J Dairy Res*, 30, 171–189.

WILLIAMS R P W, D'ATH L, AUGUSTIN M A (2005), 'Production of calcium-fortified milk powders using soluble calcium salts', *Lait*, 85, 369–381.

WOLFSCHOON-POMBO A F (1997), 'Influence of calcium chloride addition to milk on the cheese yield', *Int Dairy J*, 7, 249–254.

WU C H, LO Y S, LEE Y H, LIN T I (1995), 'Capillary electrophoretic determination of organic acids with indirect detection', *J Chromat A*, 716, 291–301.

ZOON P, VAN VILET T, WALSTRA P (1989), 'Rheological properties of rennet-induced skim milk gels. 4. The effect of pH and NaCl', *Neth Milk Dairy J*, 43, 17–34.

ZUCCHETTI S, CONTARINI G (1993), 'AAS determination of calcium, sodium, and potassium in dairy products using TCA for extraction', *Atomic Spectr*, 14, 60–64.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

# 9

# Improving the level of vitamins in milk

**B. Graulet, INRA, France**

**Abstract:** Milk is essential in human nutrition at birth but also after weaning due to milk and dairy products consumption. Bovine milk makes a significant contribution to the reference intake for several micronutrients: vitamins A (15–20%), $B_2$ (60–80%) and $B_{12}$ (90%), but all the other vitamins are also present. Clinical deficiencies in vitamins are more controlled today and some of them have almost disappeared. However, pregnant women and breast-fed babies remain at risk. A positive relation between suboptimal vitamin intakes and the prevalence of chronic diseases in the population has been observed. The increase in milk nutritional quality could help to optimize the dietary intakes of vitamins but complementary studies are needed to control precisely the vitamin composition in milk, especially for vitamins D, K and group B.

**Key words:** vitamin, milk, requirements, dairy cow.

IP Address: 129.132.208.100

## 9.1 Introduction

Milk is an essential component in animal nutrition due to its exclusive situation at birth. However, in adult human nutrition, milk and dairy products keep a highly significant position in certain geographical areas of the world, depending on socio-cultural behaviours and feeding habits. For example, inhabitants of Iceland or Finland drink about 180 kg of milk per capita per year, whereas this value is lower than 50 kg in the Far East (Haug *et al.*, 2007). The current general trend in western countries is a reduction of milk and dairy products consumption as a consequence of the negative effects attributed to saturated fatty acids on

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

heart diseases and obesity. But it should be emphasized that bovine milk is also an essential source of some micronutrients, especially vitamins such as retinol (vitamin A), riboflavin (vitamin $B_2$) and cobalamins (vitamin $B_{12}$) as suggested by the relative contribution of milk in their recommended dietary allowances (RDA) (Haug *et al.*, 2007). Moreover, due to its rather complete composition in vitamin micronutrients, among others, the milk contribution to the human diet could have benefits not only for health, but also for metabolic regulation, with its impact depending on nutritional status (Smilowitz *et al.*, 2005).

Vitamins are organic substances usually classified by their main physico-chemical property, i.e. their solubility. Fat-soluble vitamins are vitamins A, D, E and K, and water-soluble vitamins are those belonging to the B group plus ascorbic acid (vitamin C). In general, under each micronutrient called 'vitamin' is found a small family (less than 10 members) of molecularly related compounds. It can be noted that at least one member of each vitamin family is usually found in milk, although sometimes at a very low concentration. The discrepancies between concentrations of milk vitamins are the result of the combination of their specific origin (diet, microbial synthesis in the gastro-intestinal tract, endogenous synthesis by the animal tissues) that conditions their availability for the cow, their metabolism by the animal (storage and mobilization, endogenous activities, transfer to milk) and variation factors (composition of the diet, physiological status or health, for example).

Historically, vitamins were discovered and studied as factors for which nutritional deficiencies caused specific diseases: vitamin C and scurvy, vitamin $B_3$ and pellagra, vitamin D and rickets. Today, the main diseases resulting from clinical vitamin deficiencies have been identified and characterized and a RDA has been proposed for each vitamin to give a reference for the dietary intakes required to avoid clinical deficiency symptoms. However, studies on the biological properties of vitamins and their mechanisms of action are still being carried out because of their potential link to chronic diseases such as osteoporosis, diabetes, obesity or certain forms of cancer. This hypothesis is sustained by results from observational studies. For example, vitamin K intake has been associated with a lower risk of hip fracture (recently reviewed by Shea and Booth, 2008). Consequently, dietary reference intakes may have to be viewed with caution since they only represent a recommendation estimated on the basis of our actual knowledge. Elsewhere, it was reported from the SUVIMAX study that vitamin D status in 14% of healthy subjects in France did not meet the lower recommended value (Chapuy *et al.*, 1997). Thus, vitamin dietary intakes and the proposal of food ingredients (such as milk and dairy products) with the optimal nutritional value are still a question of interest. The improvement of the nutritional quality of milk can be achieved through natural means by the optimization of the diet given to dairy cows, or through technological processes such as milk fortification. Both ways are explored and could lead, through their combination, to the optimal nutritional quality of milk.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

## 9.2 Naturally occurring vitamins in cow's milk

### 9.2.1 Lipophilic vitamins

*Vitamin A*

Vitamin A is a group of several related molecules with a common molecular structure composed of two isoprene motifs and a terminal ring. The generic term 'vitamin A' is used for compounds containing a $\beta$-ionone moiety (other than carotenoids) with the biological activity of all-*trans* retinol. All-*trans* retinol (alcohol form, Fig. 9.1), 11-*cis* retinal (aldehyde form), retinoic acid (carboxylic acid form) and its esters are the most well-known members of the vitamin A family. All-*trans* retinol is generally considered as the main vitamin A form; it is also the sole vitamin A component naturally occurring in bovine milk. However, provitamins A belonging to the carotenoid family are generally present in milk as a result of transfer from the cow's diet.

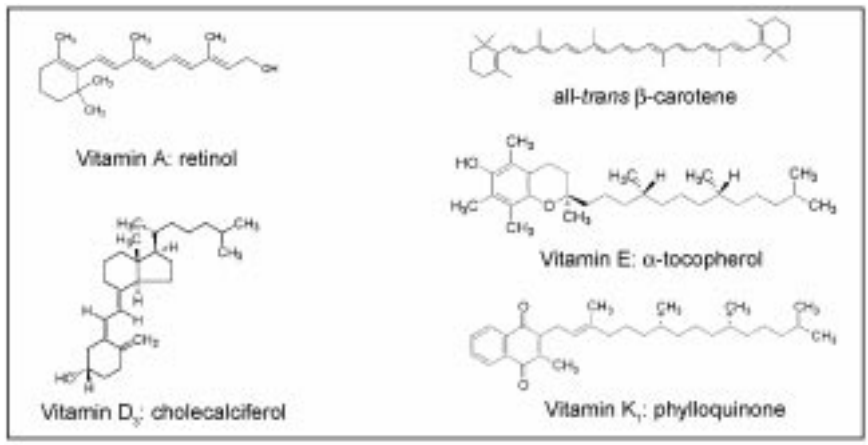
Carotenoids are liposoluble pigments found mainly in plants (and in micro-organisms). The carotenoid family is composed of carotenes (among them, all-*trans* $\beta$-carotene; Fig. 9.1) and xanthophylls (the oxygenated forms). They are characterized by a linear polyisoprene structure with conjugated double bonds which can be cyclized in its extremities. Retinol and related compounds with vitamin A activity are synthesized by animals from more than 50 carotenes that possess variable provitamin A activity. However, all-*trans* $\beta$-carotene is the carotenoid that possesses the highest vitamin A activity. Through symmetric cleavage in the enterocytes or in several other tissues such as liver, all-*trans* $\beta$-carotene is processed to two molecules of retinal by the $\beta$-carotene 15,15′ monooxygenase activity, then reduced to retinol. All-*trans* $\beta$-carotene is also the main carotenoid found in bovine milk (Nozière *et al.*, 2006). Total milk vitamin A activity would have been considered as the sum of retinol and provitamin A carotenes.

IP Address: 129.132.208.100

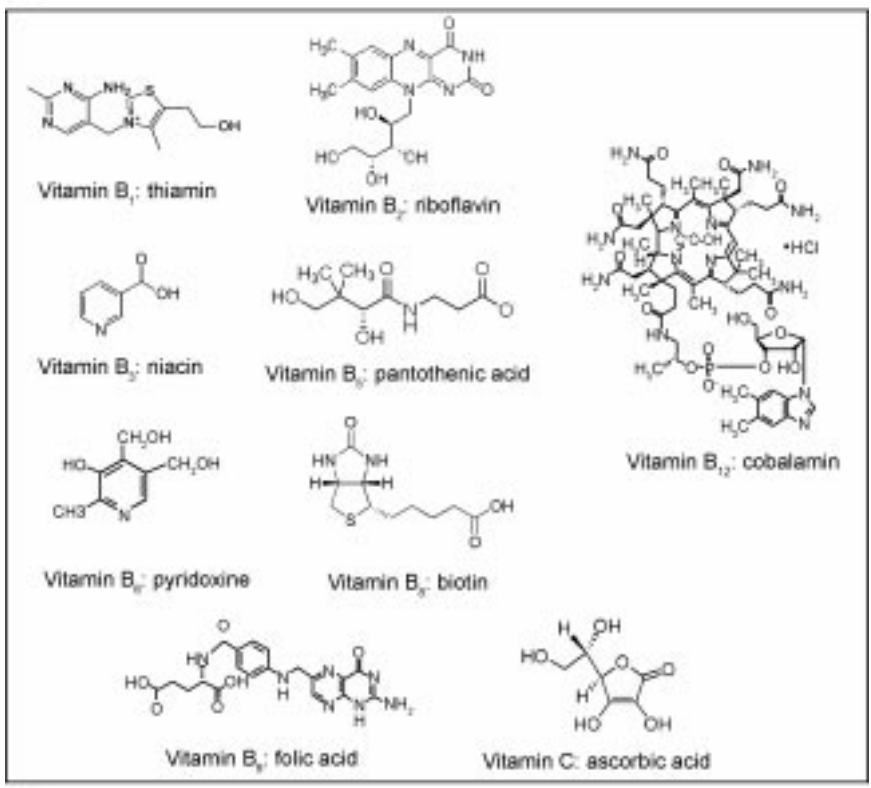
Fish liver oils (especially from halibut, shark or cod) are by far the richest sources of vitamin A; however, they do not play a great part in human nutrition. Products from ruminants are the alternative sources, providing 150 $\mu$g retinol/g of beef or sheep liver and 4–14 $\mu$g retinol/g for milk fat. Concerning poultry, egg yolk contains around 4–9 $\mu$g retinol/g (EFSA, 2008). Other foods, such as meat, kidneys or fish flesh, are not significant sources of vitamin A. In industrialized countries, half of the daily intake comes from preformed vitamin A, whereas the second half comes from carotenoids in foodstuffs from plant origin (FAO/WHO, 1988; EFSA, 2008). Recommended dietary allowances for vitamin A for adults are 600 and 900 $\mu$g retinol equivalent per day for men and 500 and 700 $\mu$g per day for women, according to the FAO/WHO data (1988) and the US National Academy of Sciences (Dietary Reference Intakes, 2001), respectively. Higher intakes are recommended for gestating (600–770 $\mu$g/d) or lactating (800–1300 $\mu$g/d) women. The relative participation of milk and dairy products in the daily contribution of preformed vitamin A to consumers varies according to nutritional habits, especially with regard to liver consumption. In Europe, for example, liver is the major source (60–80%) in France, Greece, Italy and Spain, whereas dairy products and milk

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com




(a)




(b)

**Fig. 9.1** (a) Liposoluble and (b) hydrosoluble vitamins and pseudo-vitamins in milk.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

provide 45–60% of the dairy intake in Germany, the Netherlands, Norway and Sweden (EFSA, 2008).

Due to its numerous functions, vitamin A has been extensively studied and is currently the vitamin for which cellular and molecular pathways are the most known. In the eyes, as 11-*cis* retinal, vitamin A is combined to different forms of opsin in the retina to allow vision in darkness, evaluation of brightness, or the differentiation of blue, red and green colours. In other tissues, mainly as retinoic acid (all-*trans* and 9-*cis* isomers), vitamin A follows a special mode of action, which is closer to a hormone (such as steroid or thyroid) than to other vitamins. Indeed, retinoid functions are mainly exerted through nuclear receptor pathways (RARs and RXRs). Several hundreds of genes participating in cell differentiation, embryogenesis, immune function, reproduction or growth, nervous system regulation (dopaminergic signalization) or intercellular communication were demonstrated to be regulated by retinoids.

The first effect of vitamin A deprivation is xerophthalmia, characterized by dryness of the eye epithelium and degradation of vision in darkness. Other symptoms of prolonged vitamin A deprivation are growth slowdown, anaemia (likely through an effect on iron availability) and a decrease in reproductive efficiency that affects both males and females. Worldwide, the 'Global Prevalence of Vitamin A Deficiency' was reported by OMS in 1995, as the most complete study performed on this topic. According to data, 251 million infants (0 to 4 years old) in 1994 suffered from vitamin A deprivation at a subclinical level.

Conversely, it should be noted that vitamin A is one of the vitamins for which an excess can cause a hypervitaminosis, resulting in the setting by the Scientific Committee on Food of a tolerable upper intake level of 3000 $\mu$g of retinol equivalent as preformed vitamin A per day (EFSA, 2008). This situation, resulting from excessive dietary consumption of preformed vitamin A (firstly from liver and secondarily from dairy products consumption), sometimes combined with dietary supplements enriched with vitamin A, affects 1–6% of consumers in western European countries (EFSA, 2008).

IP Address: 129.132.208.100

*Vitamin D*

The group of D vitamins is composed of 30 compounds belonging to the calciferol family. The molecular skeleton possesses a sterol structure with a central hydrophobic ring. The two precursor forms are ergocalciferol (vitamin $D_2$), synthesized in plants, and cholecalciferol (vitamin $D_3$; Fig. 9.1) produced in the skin of animals through ultraviolet irradiation of 7-dehydrocholesterol. Provitamin D has to follow a two-step sequence of hydroxylations to produce, first, 25-hydroxyvitamin D in the liver, then 1,25-dihydroxyvitamin D in kidneys (and to a lesser extent in several other tissues such as brain, colon or prostate) (Holick and Chen, 2008). These latter dihydroxylated forms are the main biologically active molecules. Several other related metabolites have been characterized but they have very low activity and rapid clearance (DeLuca, 2004).

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

Like vitamin A, vitamin D is considered to have hormone-like activity, acting via a specific nuclear receptor to achieve its different functions. The 1,25-dihydroxyvitamin D produced by kidneys is secreted in plasma to reach the intestine and bone, its two main tissue sites of action. The main role of vitamin D is to regulate calcium availability in the whole body. This is achieved through (1) the stimulation of calcium absorption in the intestine, (2) the mobilization of the osteoclastic activity (calcium resorption) in bones, and (3) calcium reabsorption at the kidney level (DeLuca, 2004). On the other hand, in other tissues, vitamin D is also considered as a regulator of the expression of more than 200 genes implicated in cell growth, differentiation and immunity. Finally, 1,25-dihydroxyvitamin $D_3$ was also demonstrated to act on cancer cells by inhibiting their growth and inducing their maturation (Holick and Chen, 2008).

Under some circumstances, sunlight-induced cutaneous synthesis of vitamin D can be impaired and, therefore, its dietary intake has to be sufficient to cover daily requirements. In general, it is considered that vitamin D is poorly provided by food, since it is usually not found in plants and is in low abundance in animal products. Vitamin D content in foods is currently of concern to the US Department of Agriculture (Holden *et al.*, 2008), since the available databases are rather poor regarding analytical determinations in foods, which also do not discriminate between $D_2$ or $D_3$ forms, for example. Taken together, it limits the estimation of the dietary intakes and of their importance for dairy requirements. In a first approach, foods with the naturally highest vitamin D content would be sea products (fish and shellfish). For example, a portion of wild salmon can contain up to 1000 IU/100 g (Holick and Chen, 2008). By comparison, in bovine whole milk (3.25% fat), the mean vitamin D content is 400 IU/L ($n = 12$), according to the USDA Food Composition Data (2009). However, these values are largely higher than reported in the scientific literature. This may be due to analytical methods, data treatments, and supplementation of the cow's diet by vitamin D.

IP Address: 129.132.208.100

Several works have reported a more complete composition of vitamin D in milk and affected the relative vitamin D activity of each calciferol-derived compound. According to authors, concentrations varied from 43 to 322 ng/L for vitamin $D_3$, from 145 to 685 ng/L for 25-hydroxyvitamin $D_3$, from 4.2 to 5.4 ng/L for 1,25-dihydroxyvitamin $D_3$, and from 27 to 45 ng/L for 24,25-dihydroxy-vitamin $D_3$ when cows received daily intakes between 4000 and 40,000 IU per day (Reeve *et al.*, 1982; Hollis *et al.*, 1981). The corresponding vitamin D activity was estimated to be between 27 and 47 IU/L in bovine milk. It is thus clear that, without being fortified by vitamin D addition, milk cannot be considered as a primary source of this vitamin for the consumer, since the adequate intakes (AI) would be in the range of 200–1000 IU according to physiological status (Dietary Reference Intakes, 1997; Weaver and Fleet, 2004; Holick and Chen, 2008). As a result of limited sun exposure and low dietary intake, one adult out of seven (14%) was found to be vitamin D-deficient in the French study SUVIMAX with a mean 3.4 $\mu$g ingested per day out of the recommended 10 $\mu$g (Chapuy *et al.*, 1997). Moreover, several factors would

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

increase the risk of deficiency such as ageing, season, increased skin pigmentation, sunscreen utilization, obesity, or several medications (Holick and Chen, 2008). The main consequences of vitamin D deficiency are growth retardation and rickets in children, increased osteopenia, osteoporosis and risk of bone failure, and muscle weakness in adults (Holick and Chen, 2008).

*Vitamin E*

The small vitamin E family is composed of eight naturally occurring components sharing a common structure: a chromanol ring with a phytyl $C_{16}$ side chain (Fig. 9.1). For the first group of four components, i.e. the tocopherols, the side chain is saturated, whereas for the other four components of the family, i.e. the tocotrienols, three double bonds are spread along the side chain (Bjorneboe *et al.*, 1990). For both groups, four different molecular forms exist ($\alpha$, $\beta$, $\delta$, and $\gamma$), according to the number and position of methyl substitutions on the phenolic ring: three methyls for $\alpha$, two methyls for $\gamma$ and $\beta$, and only one for $\delta$-tocopherol and tocotrienol. Among the eight components, $\alpha$-tocopherol usually predominates and it is thus the vitamin E form with the highest biological activity, which consists primarily as an antioxidant (Debier *et al.*, 2005). In the molecule, the side chain allows the efficient incorporation in biomembranes, and the hydroxyl function in the phenolic ring is the active site for free radical scavenging and protection of lipids from peroxidation (Bjorneboe *et al.*, 1990). Vitamin E is the most important liposoluble antioxidant for human health. It protects polyunsaturated fatty acids from oxidation in cell membranes and in plasma lipoproteins. This is especially important in newborns for proper neural development and functions and it also helps to prevent the development of degenerative diseases in adults (Bramley *et al.*, 2000; Debier *et al.*, 2005). Moreover, $\alpha$-tocopherol participates in maintaining the integrity of fat globule membranes in milk (Baldi and Pinotti, 2008). Vitamin E also improves immune system action at the cellular level (neutrophiles and macrophages) and prevents inflammatory conditions (Baldi, 2005).

The main vitamin E sources for human nutrition are mostly plant oils (wheatgerm, sunflower, rapeseed, groundnut, olive), cereals (wheat, barley), nuts (almonds), green vegetables (spinach, cabbage) and fruits (blackberries, tomatoes, avocado, blackcurrants) (Bramley *et al.*, 2000). Animal products are relatively low in vitamin E and concentrations reported in bovine milk vary between 0.2 and 1.0 mg/L (Baldi, 2005; USDA Food Composition Data, 2009). $\alpha$-Tocopherol is the main form of vitamin E in cow's milk (84–92%), the others being $\gamma$-tocopherol and $\alpha$-tocotrienol (Baldi, 2005). Milk and dairy products represent only a minor part in the recommended daily intakes (15 mg/d for adults; Dietary Reference Intakes, 2000) that are usually easily covered through diet.

Experimental vitamin E deficiencies performed on laboratory animals have shown reduction in reproduction efficiency, muscular dystrophy, exudative diathesis, megaloblastesis, pulmonary degeneration, nephrosis, and liver necrosis. (Bjorneboe *et al.*, 1990; Bramley *et al.*, 2000). However, in humans,

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

deficiencies are more often consequences of pathological situations such as lipid malabsorption syndromes like abetalipoproteinemia (Bramley *et al.*, 2000).

*Vitamin K*

Vitamin K is the fourth and lesser known group of fat-soluble vitamins sharing the same basic structure made of a 2-methyl-1,4-naphthoquinone ring with a lateral carbon chain linked at the 3-position to the ring. Members of the vitamin K family differ by the structure of the side chain. Vitamin $K_1$, also called phylloquinone, possesses a $C_{20}$ phytyl chain (Fig. 9.1), whereas in the group of vitamins $K_2$, the menaquinones (MK-*n*), the side chain is composed of a variable number ($n = 4$ to 13) of isoprenyl units. Vitamins $K_1$ and $K_2$ have different biological origins, since phylloquinone is the only important form synthesized by plants, and the menaquinones, with the exception of MK-4, are produced by bacteria in the digestive tract of humans and some other animals (Van Winckel *et al.*, 2009). Indeed, MK-4 synthesis is possible in tissues from dietary phylloquinone or menadione (also called vitamin $K_3$), the latter being a dietary supplement used in animal husbandry and also a metabolic intermediate in vitamin K processing in animals (Okano *et al.*, 2008).

Vitamin K was first discovered in the 1930s through its anti-haemorrhagic properties. It is now well known that vitamin K acts as a cofactor for the enzyme $\gamma$-glutamyl-carboxylase. This enzyme, located in the endoplasmic reticulum, is responsible for the post-translational conversion of glutamate residues into $\gamma$-carboxyglutamates in some proteins during their secretion. These carboxyglutamate residues are calcium binding groups, essential for the activity of the proteins in which they are found. It was considered first that vitamin K-dependent $\gamma$-carboxyglutamate proteins had restricted expression and distribution among tissues, but it is now well known that vitamin K participates in haemostasis through coagulation factors II (prothrombin), VII, IX and X, and through proteins C and S (feedback mechanism) produced by the liver, in calcium homeostasis (via osteocalcin and the Matrix-Gla protein), inhibits apoptosis by regulating GAS-6 activity, and regulates signal transduction and growth development (Berkner, 2005; Van Winckel *et al.*, 2009). Moreover, vitamins $K_1$ and MK-4 also have antioxidant properties protecting cerebral development. Finally, MK-4 was also reported to have specific activities mediated by gene transcription regulation via the sterol and xenobiotic receptor SXR, such as the inhibition of tumour cell proliferation (Suhara *et al.*, 2009).

Phylloquinone is the principal dietary form of vitamin K found mainly in green leafy vegetables (several hundred $\mu$g/100 g) and also in non-leafy vegetables and in vegetable oils. Bovine milk contains low amounts of phylloquinone (0.6 $\mu$g/100 g), MK-4 and very low concentrations of other MKs. In dairy products, such as cheeses, significant concentrations of MK-8 and MK-9 are observable (5–10 $\mu$g and 10–20 $\mu$g/100 g, respectively) resulting from bacterial synthesis during the fermentation process (Shearer *et al.*, 1996).

The RDA for vitamin K has been established to be 1 $\mu$g/kg of body weight per day on the basis of its anticoagulant activity. The resulting recommendations

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

are 90 $\mu$g/d for women and 120 $\mu$g/d for men (Van Winckel *et al.*, 2009). These levels seem to be easily reachable through consumption of green leafy vegetables, for example, but in fact the average daily intake estimated in a study performed in the United Kingdom was around 70 $\mu$g/d (Shearer and Bolton-Smith, 2000). The estimated mean availability of phylloquinone would be close to 80%. However, this availability probably varies depending on the vegetal matrix, and the interaction with the other components, especially the presence of fat, in the diet (Shearer *et al.*, 1996; Van Winckel *et al.*, 2009). Moreover, 60–70% of the phylloquinone oral intake is excreted within 3 days in urine (20%) and in faeces via bilary cycling (60–70%), in agreement with the rapid hepatic turnover of phylloquinone (Usui *et al.*, 1990; Shearer *et al.*, 1996). In conclusion, this implies that vitamin K stores have to be continuously provided to maintain tissue reserves and correct functions of vitamin K-dependent proteins.

The main evidence for vitamin K deficiency is the haemorrhagic disease of the newborn (less than 6 months old), which can affect breast-fed babies, sometimes causing death, as placental transfer is limited and human milk is a poor source of the vitamin. Consequently, a routine prophylaxis (oral or through intramuscular injection) with 1 mg vitamin K at birth has been adopted in most industrialized countries (Van Winckel *et al.*, 2009). Elsewhere, vitamin K deficiencies leading to a haemorrhagic risk in adults are rare (cases of fat malabsorption or cholestasis, for example) but could easily be detected by the plasma increase in proteins induced by vitamin K absence, such as under-$\gamma$-carboxylated osteocalcin or prothrombin. Much concern involves the link between vitamin K intake and skeletal health, especially from the perspective of reducing age-related bone loss. To date, the majority of the observational published studies concluded that phylloquinone intake is negatively correlated with the risk of hip fracture. However, results of randomized, controlled trials are more equivocal, since they do not usually lead to the improvement of bone health in the elderly by phylloquinone supplementation (Shea and Booth, 2008).

IP Address: 129.132.208.100

### 9.2.2 Hydrophilic vitamins

*Group B vitamins*

There are eight different families of B vitamins. The compounds are molecularly related inside a family but not between them, as we will see below. B vitamins are water-soluble and can be produced by bacteria, and most but not all can also be produced by plants (Roje, 2007). Their origin in milk is considered to be mostly from synthesis by the rumen bacteria, either *de novo* (like vitamin $B_{12}$) or from plant precursors (for others) (NRC, 2001). One other reason to treat them as a group is that the active compounds of the families are enzymatic cofactors implicated in cellular metabolism.

Vitamin $B_1$

Vitamin $B_1$, also called thiamin or aneurin (Fig. 9.1), is synthesized by plants, yeast and bacteria (Roje, 2007). In bovines, the amount of microbial thiamin

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

synthesized daily was estimated to be between 28 and 72 mg/d, that is more than equivalent to the ingested fraction (Breves *et al.*, 1981, cited by NRC, 2001). In the rumen, 48% of the thiamin from plant origin is destroyed by microorganisms (NRC, 2001). Then, thiamin is phosphorylated by enterocytes to its active form, the coenzyme thiamin-pyrophosphate (or thiamin diphosphate). In virtually all cells, it is the cofactor of enzymes involved in several oxidative decarboxylations that take part in cellular energetic metabolism at the level of the Krebs cycle (pyruvate dehydrogenase, $\alpha$-ketoglutarate dehydrogenase) and in the conversion of branched-chain $\alpha$-ketoacids (resulting from isoleucin, leucin and valin catabolism) to succinyl-coenzymeA or acetoacetate. Thiamin-pyrophosphate is also the coenzyme of the transketolase that participates in the pentose phosphate pathway (Depeint *et al.*, 2006a).

In the human diet, thiamin is mostly found in cereals (especially wholegrain and fortified cereals) and pork meat (Allen, 2003). The thiamin concentration reported in whole cow's milk is 0.44 mg/L (USDA Food Composition Data, 2009) and the average value in human milk is around 0.21 mg/L (Allen, 2003). The RDA for adults is 1.2 mg/d for males and 1.1 mg/d for females (Dietary Reference Intakes, 1998). Consequently, milk is not an important source of thiamin in human nutrition. Thiamin intake in 6–8 month old, milk-fed children would not exceed 50% of their daily requirements (Lutter and Rivera, 2003). Babies and young milk-fed children are at-risk subjects among the population, since a reduction in thiamin dietary intake by the mother quickly results in infantile thiamin deficiency symptoms (Allen, 2003). Recently, it led to a severe crisis (from 4 April to 13 July 2004) in the overseas French area of Mayotte, where 32 babies were diagnosed for beriberi (62% lethality), the main known disease resulting from thiamin deficiency (Institut National de Veille Sanitaire, Rapport d'Investigation, 2004). Symptoms of clinical thiamin deficiency in the child are peripheral neuropathy, encephalopathy and cardiac failure. Fortunately, such situations are limited today to undernourished women fed a polished rice-based diet or people in refugee camps and can be avoided by supplementations and prevention programmes (Allen, 2003). In adults, long-lasting thiamin deficiency induces first a loss of appetite until inanition that leads to cardiovascular and neurological troubles. People with diseases related to food ingestion, with excessive alcohol consumption or living in nutritionally deprived conditions are still at risk in the population (Allen, 2003).

IP Address: 129.132.208.100

### Vitamin $B_2$

Vitamin $B_2$ is better known as riboflavin but is also referred to as lactoflavin, vitamin G or lactochrome (Fig. 9.1). Like thiamin, riboflavin is synthesized by plants and microorganisms, and mammals are dependent on dietary intakes (Roje, 2007). In ruminants, dietary riboflavin is almost totally degraded by microorganisms (NRC, 2001; Santschi *et al.*, 2005). Consequently, the riboflavin present in cow's milk results from rumen synthesis. After absorption in the proximal small intestine, riboflavin is activated in cells into flavin mononucleotide (FMN), then converted into flavin adenine dinucleotide (FAD). They

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

are both the main biologically active forms of vitamin $B_2$ (Powers, 2003). Together, they participate as prosthetic groups of numerous enzymes (oxidases, reductases and dehydrogenases) called flavoproteins, in oxidoreduction reactions essential for cell life (for review of an exhaustive list, see Depeint *et al.*, 2006a). For example, FMN and FAD participate in electron transfer in the mitochondrial respiration pathway, in the initiation of fatty acid catabolism by $\beta$-oxidation, in the Krebs cycle, as cellular antioxidant protectants (through the glutathione reductase and the glutathione peroxidase) and also in the metabolism of purine bases and amino acids (Haug *et al.*, 2007).

In the human diet, most plant and animal products are sources of riboflavin. However, major riboflavin intakes come from animal products like eggs, lean meat and milk (Allen, 2003; Depeint *et al.*, 2006a). The riboflavin concentration in whole cow's milk reported in the tables is $1.83 \pm 0.02$ mg/L (USDA Food Composition Data, 2009) and the average value in human milk is considerably lower, around 0.35 mg/L in well-nourished women (Allen, 2003). The RDA for adults is 1.3 mg/d for men and 1.1 mg/d for women (Dietary Reference Intakes, 1998). The calculated contribution of milk in the reference intake is between 60 and 80% (Haug *et al.*, 2007). Thus, among water-soluble vitamins, riboflavin has a unique position, since it is the sole vitamin for which milk and dairy products are the greatest contributors to its intake, especially in western countries (Powers, 2003). In other countries, contributions from green vegetables increase proportionally to the decrease of that due to milk (Allen, 2003). Riboflavin deficiencies are observed usually when dietary intake of animal products is low. It has a high prevalence among lactating women and the elderly in Guatemala, rural children in Mexico and also people living in China (Allen, 2003; Powers, 2003). The corresponding disease is ariboflavinosis, which has typical symptoms such as lip and tongue inflammations. However, due to its crucial role in cellular metabolism, numerous adverse effects occur following riboflavin deficiencies such as anaemia through interference with iron handling and possibly cancer, and cardiovascular diseases perhaps by interfering with folate-homocystein metabolism (Powers, 2003; Depeint *et al.*, 2006a).

IP Address: 129.132.208.100

### The vitamin $B_3$ group

The vitamin $B_3$ group is mainly composed of nicotinic acid (also named niacin) and nicotinamide, the latter possessing the biological activity. The term niacin refers to a group of pyridine–carboxylic acids and their derivatives (Fig. 9.1). This vitamin has also been called vitamin PP for 'pellagra preventive', since pellagra is a consequence of the deficiency. Niacin is a metabolic precursor of nicotinamide adenine dinucleotide ($NAD^+$) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). However, the pyridine ring of $NAD^+$ can also be synthesized *de novo* from tryptophan in animals and bacteria (Roje, 2007). $NAD^+$ and $NADP^+$ are cofactors of more than several hundred reactions, including mitochondrial respiration, glycolysis, and $\beta$-oxidation, which are dependent on the dietary intake of niacin (in one form or another) and/or tryptophan. In the ruminant, it is likely that bacterial niacin covers the animal

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

requirements and provides the most part of its secretion in milk, since the microbial degradation of the dietary intakes seems extensive (NRC, 2001; Santschi *et al.*, 2005). In the intestine, niacin is absorbed as nicotinamide, which is also the circulating form of vitamin $B_3$. In tissue cells, nicotinamide is metabolized to $NAD^+$ and $NADP^+$. With riboflavin, niacin is one of the two group-B vitamins that are cofactors in oxidoreduction reactions. Among others, $NAD^+$ functions as an electron carrier for ATP production by cells. $NADP^+$ is a hydrogen donor for fatty acid, sterol or pentose biosynthesis, and also in the process of glutathione regeneration in the antioxidant protection of the cells (Depeint *et al.*, 2006a).

In human nutrition, the main sources of niacin are meat (above all liver, fish and poultry), cereal-based products including bread, milk and green leafy vegetables. In industrialized countries, cereals (fortified wholegrain or enriched) are the main sources of niacin (Allen, 2003). The reference niacin value in cow's milk is $1.07 \pm 0.03$ mg/L (USDA Food Composition Data, 2009), whereas the average concentration reported in human milk is 1.8 mg niacin equivalent/L (considering that 60 mg of tryptophan allows the synthesis of 1 mg of niacin; Allen, 2003). The RDA value was determined as between 12 and 16 mg/d for males and 12 to 14 mg/d for females (from nine to more than 70 years old; Dietary Reference Intakes, 1998). Once the intakes are below the recommended values (rarely, but in case of extreme alcoholism, anorexia, or seasonally in some at-risk countries), symptoms of deficiency occur: pellagra is a chronic wasting disease initially thought to be from infectious origin and characterized by dermatitis, dementia and diarrhoea. It is characterized by a rash pigmentation linked to sunlight exposure, red tongue, gastrointestinal disturbances and neurological abnormalities. However, at the beginning of the nineteenth century, the link between pellagra and a diet based on maize, naturally poor in tryptophan and absorbable niacin, was established (Allen, 2003; Bogan and Brenner, 2008).

IP Address: 129.132.208.100

### Vitamin $B_5$ – pantothenic acid

Vitamin $B_5$ relates to only one compound, pantothenic acid (Fig. 9.1), and not to a family. Like the other group B vitamins already presented in this chapter, vitamin $B_5$ can be synthesized by plants and by microorganisms, but not by animals (Roje, 2007). In the ruminant species, rumen microbial synthesis largely overcomes the dietary intake; however, as the estimated degradation is not total (78%; NRC, 2001), it is likely that milk vitamin $B_5$ is a combination of dietary intake and rumen synthesis.

Vitamin $B_5$ is the precursor of coenzyme A which plays a central role in numerous fundamental reactions: fatty acid oxidation, amino acid catabolism, acetylcholine synthesis, heme synthesis and as a prosthetic group in the Krebs cycle. Consequently, pantothenic acid is provided largely by food in humans, not only as pantothenic acid *per se*, but also as coenzyme A. The latter is present mainly in animal organs, egg yolks, peanuts and beans, lean meats, milk ($3.62 \pm 0.03$ mg/L; USDA Food Composition Data, 2009), potatoes and green leafy vegetables (Depeint *et al.*, 2006a). Thus, a dietary deficiency of vitamin $B_5$

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

is extremely rare and it seems that it could only affect breast-fed babies of mothers fed a deficient diet (based mainly on refined cereals, for example) but not healthy adults. The mean concentrations reported in human milk are 2.2 mg/L (Allen, 2003) but with a large variability (from 0.7 to 4.5 mg/L), and higher values have already been observed (6.7 mg/L) in mothers with high intakes, since a good correlation has been observed between pantothenic acid intake and milk secretion (Johnston *et al.*, 1981). As a consequence of the absence of dietary deficiency in humans, there is no RDA value but only a proposition of adequate intake (AI) at 5 mg/d for normal adults (Dietary Reference Intakes, 1998).

### The vitamin $B_6$ group

Vitamins $B_6$ are a group of three compounds: pyridoxine, the three-alcohol form (Fig. 9.1), pyridoxal, for which one alcohol was oxidized into an aldhehyde function, and pyridoxamine where the aldehyde is replaced by a primary amine substitution. All three are precursors of pyridoxal-5′-phosphate (P5P), a cofactor for numerous reactions involved in amino acid metabolism. The reactions involve transaminases that participate in the catabolism of amino acids by the urea cycle, decarboxylases in heme synthesis from glycine, enzymes acting in cysteine, glycine or taurine production, in activation of tetrahydrofolate by serine for the remethylation of homocysteine, and in tryptophan metabolism to NAD (Depeint *et al.*, 2006b). As reported for most of the other group B vitamins, vitamin $B_6$ can be synthesized by plants and microorganisms, but studies have indicated that the net production by the rumen is limited (Santschi *et al.*, 2005; Schwab *et al.*, 2006). Consequently, the vitamin $B_6$ secreted in cow's milk would be closely linked to the animal's dietary intake. However, mean concentrations available are 0.36 mg/L and the variability seems extremely limited (USDA Food Composition Data, 2009). In human milk, values are close to 0.13 mg/L but can decrease to less than 0.068 mg/L (Allen, 2003). It should be noted here that vitamin $B_6$ is mainly the pyridoxal form in both cow's and human milk (Vanderslice *et al.*, 1983).

IP Address: 129.132.208.100

Human cells can synthesize the P5P from the three $B_6$ vitamins but not *de novo* and they consequently must be provided through the diet. In spite of the fact that they are not directly absorbable, dietary phosphorylated forms of the compounds are, by themselves, good sources of vitamin $B_6$ since the human intestine produces phosphatases that are able to hydrolyse them before absorption (Depeint *et al.*, 2006b; Roje, 2007). In general, pyridoxin, pyridoxal and P5P are the major forms encountered in the human diet. They are mainly present in vegetables, wholegrain cereals, nuts and muscle meats. However, during thermal processing or storage, the formation of a Schiff base between pyridoxal and lysine residues occurs and limits vitamin $B_6$ availability.

Clinical vitamin $B_6$ deficiencies are rarely encountered because of its widespread occurrence in foods, as for pantothenic acid. The clinical symptoms linked to vitamin $B_6$ deficiency are epileptic seizures, anaemia, renal failure and dermatitis (Depeint *et al.*, 2006b). However, it was established that 10% of the

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

US population ingests below half the recommended value (Depeint *et al.*, 2006b) of 1.3 to 1.7 mg/d for adult males and 1.2 to 1.5 mg/d for females (Dietary Reference Intakes, 1998). Chronic subclinical deficiency would affect the overall population in industrialized and developing countries and be associated with an increased risk of cardiovascular diseases, stroke, cancers (colon), and Alzheimer's disease (Depeint *et al.*, 2006b; Roje, 2007).

### Vitamin $B_8$

Biotin (Fig. 9.1) is the compound behind all the following names: vitamin $B_7$, vitamin $B_8$, vitamin H or coenzyme R. It is synthesized by plants and bacteria from alanine and pimelic acid (Roje, 2007). Thus, biotin is usually produced by rumen microorganisms; moreover, dietary intakes are poorly degraded and the level of biotin secretion in milk is quantitatively related to its intake (NRC, 2001). Biotin is the prosthetic group of five cellular enzymes acting in carboxyl-group transfers, mostly in fatty acid homeostasis but also in leucine catabolism, gluconeogenesis and vitamin $B_9$ and $B_{12}$ activities. The enzymes are acetyl-coenzymeA carboxylases, 3-methylcrotonyl-coenzymeA carboxylase, propionyl-coenzymeA carboxylase and pyruvate carboxylase (Hassan and Zempleni, 2006; Depeint *et al.*, 2006a; Roje, 2007). Independently of these metabolic activities, biotin plays a major role in the regulation of gene expression. Indeed, it is now well established that biotin is covalently bound to specific lysine residues in histones and would then regulate the expression of more than 2000 genes in human cells (Hassan and Zempleni, 2006).

Biotin deficiency is extremely rare in humans due to its high prevalence in food such as cow's milk (8 $\mu$g/L, which is similar to the value reported for human milk: Allen, 2003), liver, egg yolk, vegetables and fruits, and meat products. As is the case for pantothenic acid, some values of AI are proposed: 20 to 30 $\mu$g/d for adults, with the exception of lactating women (35 $\mu$g/d) (Dietary Reference Intakes, 1998). However, during pregnancy 40% of women would have a biotin deficiency (Depeint *et al.*, 2006a). Elsewhere, the only reported clinical case of dietary deficiency (neurological symptoms, hair loss and red facial rash, among others) was the result of the excessive consumption of raw egg white which contains high levels of avidine, a natural ligand of biotin. Experimentally, it was demonstrated in laboratory animals that low bioavailable biotin levels could have teratogenic effects (Allen, 2003; Depeint *et al.*, 2006a).

### Vitamin $B_9$

Folates are a class of compounds with a chemical structure and biological activities similar to those of folic acid (pteroyl-L-glutamic acid; Fig. 9.1). In nature, the molecule is present as dihydro- or tetrahydrofolate (THF), is substituted by different kinds of one carbon units (methyl, formyl, methenyl, methylene and formimino), and possesses five to seven glutamate residues in the side chain (polyglutamate form). Folic acid is synthesized by plants and microorganisms (Roje, 2007). In the dairy cow, dietary folic acid is almost totally destroyed or used before leaving the rumen, suggesting that microbial synthesis

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

activity (16 to 21 mg/d; Santschi *et al.*, 2005; Schwab *et al.*, 2006) is not necessarily sufficient to cover the requirements of the cow (NRC, 2001; Girard and Matte, 2005).

Folates circulate in plasma as monoglutamylfolates (mostly 5-methyl-THF) and are subsequently elongated to polyglutamates in cells where they are trapped. However, the elongation requires first the demethylation of the 5-methyl-THF through the vitamin $B_{12}$-dependent methionine synthase. Then, glutamate residues can be added to the side chain of the folates, which become really active for one-carbon group transfer. The different forms of folates are essential for nucleic acid synthesis (purines, thymidylate, formyl-Met-tRNA) and in the methylation cycle that necessitates methionine regeneration from homocysteine before activation to *S*-adenosylmethionine, the primary methylating agent (for review, see Girard and Matte, 2005, and Depeint *et al.*, 2006b).

Main sources of folates in the human diet are grains, oranges, eggs and green vegetables (Depeint *et al.*, 2006b). Concentrations in bovine milk are between 50 and 90 $\mu$g/L, mainly as 5-methyl-THF (Forssén *et al.*, 2000; USDA Food Composition Data cited by Haug *et al.*, 2007). The corresponding value in human milk is 85 $\mu$g/L (Allen, 2003). Recommended daily intakes of folates are 400 $\mu$g/d for both men and women but this level increases to 500 $\mu$g/d in lactating women and 600 $\mu$g/d during pregnancy (Dietary Reference Intakes, 1998). On average, folates from dairy products, including milk, supply 10–15% of the daily intake in western countries, especially among youths. It is now generally assumed that folate deficiency is the most prevalent vitamin deficiency. This is the reason why the RDA was increased in 1989. Moreover, some countries such as the USA and the United Kingdom recommend daily supplements of folic acid for pregnant women (Forssén *et al.*, 2000). The first symptom of folate deficiency is the transformation of the plasma erythrocytes and bone marrow cells that enlarge as a result of the reduction in DNA, RNA and protein synthesis. This phenomenon is called megaloblastic anaemia. It can occur in cases of malnutrition, severe alcoholism or diseases that alter absorption efficacy (Forssén *et al.*, 2000).

In pregnant women, the suboptimal status of the mother leads to premature birth, low birth weight, neural tube defects with spina bifida, and occasionally with anencephaly for the babies. However, as this latter defect results from an early event in the development of embryos, women should have sufficient folate intakes even before conception. What is really dangerous here is that it concerns not only women with low dietary folate intakes or specific conditions reported above. As soon as conception has occurred, and throughout the pregnancy, the folate requirements will increase and they consequently could not have been provided by food. Under these conditions, folic acid supplementation reduces the risk of foetal troubles by 50–75% (Hathcock, 1997; Forssén *et al.*, 2000). Folate deficiency also limits methionine regeneration and consequently induces an increase in plasma homocysteine concentration, which is well known as an independent risk factor of coronary heart disease. Epidemiological studies have

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

also shown the positive relation between high folate levels and the reduction in cancer risk (Forssén *et al.*, 2000).

### Vitamin $B_{12}$

Vitamin $B_{12}$ is something special among the vitamins: it is synthesized neither by plants nor by animals. Indeed, only microorganisms (bacteria, algae, etc.) are able to produce cobalamins, which are a small group among the corrinoids. Corrinoids are cyclic molecules containing a core structure, the corrin part, which is identical to heme except for two things: the central metal ion is cobalt and not iron, and one of the internal alpha methene bridges is missing in the corrin nucleus. Not all the corrinoids possess vitamin $B_{12}$ biological activity in humans. Bacteria produce numerous corrinoids, the vitamins $B_{12}$ (cobalamins) and their analogues, the latter being usable by microorganisms but not by mammals. Human tissues are able to use specifically only the cobalamins that are composed of the corrin ring plus an aminopropanol residue, a sugar, a nucleotide and an adduct linked to the cobalt atom (Fig. 9.1). The chemical nature of the adduct conditions the cobalamin to be biologically active in humans: hydroxocobalamin, aquacobalamin, 5′-desoxyadenosylcobalamin and methylcobalamin. A last vitamin $B_{12}$ is cyanocobalamin, which is the industrial purified form and is not directly usable by human tissues but can be hydrolysed in cells to generate biologically active forms (Herbert, 1988).

Bacteria present in the human colon synthesize cobalamins biologically active for humans. However, the molecules are not absorbed by the colon but at the level of the small bowel (Herbert, 1988). Consequently, in human diet, cobalamins have to be exclusively supplied by the diet, essentially in feed ingredients from animal origin: milk and dairy products, meat, poultry, eggs and fish. Bovine milk provides an average of $4.4 \pm 0.3\,\mu$g vitamin $B_{12}$/L (USDA Food Composition Data, 2009), mostly hydroxocobalamin (Depeint *et al.*, 2006b). The vitamin secreted in cow's milk is synthesized by rumen microorganisms using dietary cobalt. It was estimated that dairy cattle would need 0.34 to $0.68\,\mu$g cobalamin/kg of live body weight and that rumen synthesis would cover the entire requirement (NRC, 2001). However, recently, the vitamin $B_{12}$ apparent ruminal synthesis was evaluated to be between 60 and 102 mg/d (Santschi *et al.*, 2005; Schwab *et al.*, 2005); these amounts would not be enough to meet requirements, but the latter values are probably underestimated because it is experimentally difficult to take into account the rumen utilization of cobalamins and of their absorption before the small intestine canula (Santschi *et al.*, 2005).

The human requirements have been estimated to be around $1\,\mu$g/d (Herbert, 1988; Depeint *et al.*, 2006b) but the RDA is up to $2.4\,\mu$g/d in normal adults and $2.8\,\mu$g/d in lactating women (Dietary Reference Intakes, 1998). A glass of cow's milk alone could cover 42% of adult daily requirements for vitamin $B_{12}$ (Girard and Matte, 2005).

Vitamin $B_{12}$ participates in one-carbon transfer pathways since the methylcobalamin is the cofactor of methionine synthase that catalyses the regeneration

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded From Vet-eBooks.com

of methionine from homocysteine and 5-methyl-THF (vitamin $B_9$). This reaction is important (1) for the cycle of the cellular methylation pathway because the resulting methionine can be further activated into *S*-adenosylmethionine, the primary methyl donor of cells, (2) for the reduction in homocysteine levels since it is a risk factor for coronary heart diseases, and (3) for the transformation of methyl-THF into a free form, now available for activation with other one-carbon groups for nucleic acid synthesis. Another role of vitamin $B_{12}$, as adenosyl-cobalamin, is to favour the catabolism of valine, isoleucine or odd-chain fatty acids into succinyl-coenzymeA by acting as a cofactor of the methylmalonyl-coenzymeA mutase. Cobalamin deficiency leads first to pernicious anaemia for which a classical symptom is the observation of expanded plasma erythrocytes to macrocytes. Due to the metabolic connections previously reported, cobalamin deficiency also affects folate metabolism (reduction in methionine availability, increase in homocysteine plasma concentration, hypomethylation including in DNA which could induce carcinogenesis). Moreover, it leads to an increase in methylmalonyl acid in cells and plasma and consequently, by toxicity to mitochondria, to hypoglycaemia, hyperglycinemia and hyperamonemia (Depeint *et al.*, 2006b).

Vitamin $B_{12}$ deficiency affects 10–15% of the elderly population in the USA (>60 years old), not necessarily from insufficient dietary intakes but rather from reduced absorption efficiency (Stabler *et al.*, 1997; Depeint *et al.*, 2006b). This observation is of greatest importance because of the potential link between vitamin $B_{12}$ and atherosclerosis risk. Deficiency in breast-fed children can also occur (slow growth and developmental delays) since the human milk from normal well-nourished women is 10-fold less concentrated than bovine milk (0.42 $\mu$g/L; Allen, 2003). Deficiencies were observed in several areas of the world (Latin America) but also in strict vegetarian populations in industrialized countries (Allen, 2003).

IP Address: 129.132.208.100

*Vitamin C*

Vitamin C is the pair of ascorbic and dehydroascorbic acids (Fig. 9.1). Ascorbic acid is a small molecule with a lactone ring. It is an electron donor that gives it its antioxidant property. So, it acts as a cofactor for 11 enzymes used for collagen hydroxylation, biosynthesis of carnitine or catecholamine and norepinephrine, amidation of peptide hormones, tyrosine metabolism and also monooxygenase and dioxygenase activities. Elsewhere, vitamin C also helps to protect food and plasma folates, and to absorb soluble non-heme iron.

It is synthesized in the liver of some mammals (including bovines) but not in primates due to the lack of the ultimate enzyme of the synthesis pathway. Consequently, ascorbic acid is not considered as an essential nutrient in dairy cows. However, in humans, vitamin C must be supplied by the diet to avoid deficiencies and their consequences, including scurvy, characterized by gingival changes, pain in the extremities, haemorrhagic events and ulcerations and then death. The RDA is fixed at 90 mg/d for men and 75 mg/d for women (Dietary Reference Intakes, 2000). It can be easily covered by fruit (mostly citrus and

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

tomatoes) and green-leafy vegetable consumption. Bovine milk could be a complementary source, considering that it contains between 17 and 23 mg/L of the vitamin (Graham, 1973; Hidiroglou *et al.*, 1995; Weiss, 2001; Weiss *et al.*, 2004).

## 9.3 Techniques to improve vitamin content of milk

Most studies on the supplementation of the diet of dairy cows by vitamins have been conducted to test the effects on animal performance, including production efficacy, reproduction, and improvement of immunity. However, the factors responsible for variations of the content of vitamins in milk have been relatively poorly investigated. For lipophilic vitamins, milk concentrations are linked to the transfer of vitamins from the feed ingredients or the mineral and vitamin complements to milk. Data on the factors regulating the transfer of carotenoids to milk as retinol or $\beta$-carotene have been recently reviewed and will be briefly presented (for review, see Nozière *et al.*, 2006).

Many factors have been suggested to explain the variability in retinol and carotenoids in milk. Among them are non-dietary factors such as the breed, the stage of lactation, the health status of the udder, the milk and fat yields and the genetic traits. The general trend is that retinol concentrations are less variable than those of $\beta$-carotene. More interesting are the variations linked to dietary factors, since the levels of fat-soluble vitamins A, E and $\beta$-carotene in milk are highly linked to the amounts consumed by the cows. Experimentally, the highest levels are usually found in spring and summer in cows fed at pasture with a carotenoid and vitamin E-rich grass. In the milk of cows at pasture, concentrations reach 5 to 6 $\mu$g/g fat for $\beta$-carotene and retinol (Nozière *et al.*, 2006) and 0.63 $\mu$g/mL for vitamin E (Martin *et al.*, 2004). The values decrease when the diet is based on grass silage, hay or maize silage (between 2.5 and 2.8 $\mu$g/g fat for $\beta$-carotene and retinol and 0.48 $\mu$g/mL for vitamin E) (Martin *et al.*, 2004; Nozière *et al.*, 2006). These observations can be explained by the dose–response relationship established between carotenoid or vitamin E intakes and carotenoid, retinol or vitamin E concentrations in milk; whereas $\beta$-carotene and retinol secretion seems easily limited by a saturation process, vitamin E concentrations increase linearly with the dose ingested (Calderón *et al.*, 2007). However, in real conditions at the farm, these effects are often limited due to vitamin A and E dietary supplementations to the animals that compensate for the reduction of the vitamin intakes with the conserved forages (Agabriel *et al.*, 2007). Indeed, under practical conditions of herd management, vitamin A supplementation has no effect on retinol concentration of dairy products when herds are fed at pasture but is the main factor contributing to the concentration of retinol in milk when cows receive mostly preserved forages (Lucas *et al.*, 2006). Data concerning vitamins D and K are lacking to evaluate the factors contributing to their respective milk concentrations and ways to potentially increase their secretion. However, McDermott *et al.* (1985) did not observe an increase in milk

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

vitamin D activity after dietary supplementation of cows by comparison with control cows.

Concerning the group B vitamins, a limited amount of research was originally conducted since they are available from both dietary intakes and bacterial rumen synthesis, with these two ways being considered as sufficient to cover the cow's estimated requirements. However, the productivity of cows has strongly increased in recent decades and probably the same applies to the metabolism of animals, in which group B vitamins are highly involved. Moreover, the consequences of vitamin deficiencies were revisited after the description of the large panel of effects resulting from subclinical intakes in the human population (see above). Thus, it would be interesting to improve the nutritional quality of food, including milk and dairy products, by optimizing their vitamin content, especially with liposoluble vitamins and also folates, riboflavin and cobalamins (Smilowitz *et al.*, 2005). However, in general, milk concentrations of group B vitamins seem poorly related quantitatively to their dietary intakes from feed ingredients due to their extensive degradation and synthesis in the rumen (Haug *et al.*, 2007). Recent work has been performed to estimate the behaviour of these vitamins supplied by feeds (Schwab *et al.*, 2006), or provided by supplementation in the diet or by post-ruminal infusion (Santschi *et al.*, 2005). Schwab *et al.* (2006) concluded that not only the dietary intake but also the duodenal flow and ruminal synthesis are modified by the composition of the diet, i.e. the nature of the forage and the content in non-fibre carbohydrates. Unfortunately, the effects on milk vitamin concentrations are not available in these two papers. However, when amounts of folic acid and vitamin $B_{12}$ similar to those supplied by Santschi and co-workers were fed to lactating cows, increasing secretion into milk was observed for these two vitamins (Graulet *et al.*, 2007), suggesting that a fraction of the dose bypasses degradation in the rumen. However, in these two latter experiments, high doses of supplements were given. For practical application, an increase in milk group B vitamins would require either control of the ruminal processes according to the composition of the cow's diet or the use of rumen-protected B vitamins.

IP Address: 129.132.208.100

Obviously, it is always possible to improve milk quality by modifying its composition. Such is actually the case for vitamin D but the nutritional relevance of the supplementations for the population is not always in agreement with economic benefits.

## 9.4 Conclusions

In this paper the vitamins available in cow's milk and their nutritional benefits were presented, as well as the importance of milk in the recommended daily allowance of each compound. We saw that things are really different between vitamins, some being more specific to animal products (vitamins A, $B_2$ and $B_{12}$), while others are supplied by vegetal feeds in the human diet. Data on retinol concentrations in milk are rather well known, whereas for vitamins D and K, for

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

example, we do not have a lot of data to understand the basic regulation of their secretion in milk. It is almost the same for the B vitamins that have been neglected for a very long time. In this concern, incremental data are needed to evaluate the factors that regulate (1) their synthesis and degradation in the rumen, and (2) the transfer from diet (as a purified supplement or included in the feed ingredients) to milk.

## 9.5 References

AGABRIEL C, CORNU A, JOURNAL C, SIBRA C, GROLIER P and MARTIN B, 2007. Tanker milk variability according to farm feeding practices: vitamins A and E, carotenoids, colour and terpenoids. *J Dairy Sci*, 90, 4884–4896.

ALLEN LH, 2003. B vitamins: proposed fortification levels for complementary foods for young children. *J Nutr*, 133, 3000S–3007S.

BALDI A, 2005. Vitamin E in dairy cows. *Liv Prod Sci*, 98, 117–122.

BALDI A and PINOTTI L, 2008. Lipophilic microconstituents of milk. *Adv Exp Med Biol*, 606, 109–125.

BERKNER KL, 2005. The vitamin K-dependent carboxylase. *Ann Rev Nutr*, 25, 127–149.

BJORNEBOE A, BJORNEBOE GE and DREVON CA, 1990. Absorption, transport and distribution of vitamin E. *J Nutr*, 120, 233–242.

BOGAN KL and BRENNER C, 2008. Nicotinic acid, nicotinamide, and nicotinamide riboside: a molecular evaluation of $NAD^+$ precursor vitamins in human nutrition. *Ann Rev Nutr*, 28, 115–130.

BRAMLEY PM, ELMADFA I, KAFATOS A, KELLY FJ, MANIOS Y, ROXBOROUGH HE SCHUCH W, SHEEHY PJA and WAGNER K-H, 2000. Review. Vitamin E. *J Sci Food Agr*, 80, 913–938.

BREVES G, BRANDT M, HOELLER H and ROHR K, 1981. Flow of thiamin to the duodenum in dairy cows fed different rations. *J Agric Sci* (Cambridge), 96, 587–591.

CALDERÓN SF, CHAUVEAU-DURIOT B, PRADEL P, MARTIN B, GRAULET B, DOREAU M and NOZIÈRE P, 2007. Variations in carotenoids, vitamins A and E, and color in cow's plasma and milk following a shift from hay diet to diets containing increasing levels of carotenoids and vitamin E. *J Dairy Sci*, 90, 5651–5664.

CHAPUY MC, PREZIOSI P, MAAMER M, ARNAUD S, GALAN P, HERCBERG S and MEUNIER PJ, 1997. Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporosis Intern*, 7, 439–443.

DEBIER C, POTTIER J, GOFFE C and LARONDELLE Y, 2005. Present knowledge and unexpected behaviours of vitamins A and E in colostrum and milk. *Liv Prod Sci*, 98, 135–147.

DELUCA HF, 2004. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr*, 80 (suppl.), 1689S–1696S.

DEPEINT F, ROBERT BRUCE W, SHANGARI N, MEHTA R and O'BRIEN PJ, 2006a. Mitochondrial function and toxicity: role of the B vitamin family on mitochondrial energy metabolism. *Chem-Biol Inter*, 163, 94–112.

DEPEINT F, ROBERT BRUCE W, SHANGARI N, MEHTA R and O'BRIEN PJ, 2006b. Mitochondrial function and toxicity: role of B vitamins on the one-carbon transfer pathways. *Chem-Biol Inter*, 163, 113–132.

DIETARY REFERENCE INTAKES FOR CALCIUM, PHOSPHORUS, MAGNESIUM, VITAMIN D AND FLUORIDE, 1997. Recommended Dietary Allowances, Xth ed. Washington, DC:

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

National Academy of Sciences.

DIETARY REFERENCE INTAKES FOR THIAMIN, RIBOFLAVIN, NIACIN, VITAMIN $B_6$, FOLATE, VITAMIN $B_{12}$, PANTOTHENIC ACID, BIOTIN AND CHOLINE, 1998. Recommended Dietary Allowances, Xth ed. Washington, DC: National Academy of Sciences.

DIETARY REFERENCE INTAKES FOR VITAMIN C, VITAMIN E, SELENIUM AND CAROTENOIDS, 2000. Recommended Dietary Allowances, Xth ed. Washington, DC: National Academy of Sciences.

DIETARY REFERENCE INTAKES FOR VITAMIN A, VITAMIN K, ARSENIC, BORON, CHROMIUM, COPPER, IODINE, IRON, MANGANESE, MOLYBDENUM, NICKEL, SILICON, VANADIUM AND ZINC, 2001. Recommended Dietary Allowances, Xth ed. Washington, DC: National Academy of Sciences.

EUROPEAN FOOD SAFETY AUTHORITY, 2008. Scientific opinion of the panel on additives and products or substances used in animal feed on a request from the European Commission on the consequences for the consumer of the use of vitamin A in animal nutrition. *EFSA J*, 873, 1–81.

FAO/WHO, 1988. Requirements of vitamin A, iron, folate and vitamin B12. *Food and Nutrition Series* no. 23, FAO, Rome.

FORSSÉN KM, JÄGERSTAD MI, WIGERTZ K and WITTHÖFT CM, 2000. Folates and dairy products: a critical update. *J Am College Nutr*, 19, 100S–110S.

GIRARD CL and MATTE JJ, 2005. Folic acid and vitamin $B_{12}$ requirements of dairy cows: a concept to be revised. *Liv Prod Sci*, 98, 123–133.

GRAHAM DM, 1973. Alteration of nutritive value resulting from processing and fortification of milk and dairy products. *J Dairy Sci*, 57, 738–745.

GRAULET B, MATTE JJ, DESROCHERS A, DOEPPEL L, PALIN MF and GIRARD CL, 2007. Effects of dietary supplements of folic acid and vitamin $B_{12}$ on metabolism of dairy cows in early lactation. *J Dairy Sci*, 90, 3442–3455.

HASSAN YI and ZEMPLENI J, 2006. Epigenetic regulation of chromatin structure and gene function by biotin. *J Nutr*, 136, 1763–1765.

HATHCOCK JN, 1997. Vitamins and minerals: efficacy and safety. *Am J Clin Nutr*, 66, 427–437.

HAUG A, HOSTMARK A and HARSTAD O, 2007. Bovine milk in human nutrition – a review. *Lipids in Health and Disease*, 6, 25. http://lipidworld.com/content/6/1/25

HERBERT V, 1988. Vitamin B-12: plant sources, requirements, and assay. *Am J Clin Nutr*, 48, 852–858.

HIDIROGLOU M, IVAN M and BATRA TR, 1995. Concentrations of vitamin C in plasma and milk of dairy cattle. *Ann Zootech*, 44, 339–402.

HOLDEN JM, LEMAR LE and EXLER J, 2008. Vitamin D in foods: development of the US Department of Agriculture database. *Am J Clin Nutr*, 87 (suppl.), 1092S–1096S.

HOLICK MF and CHEN TC, 2008. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr*, 87 (suppl.), 1080S–1086S.

HOLLIS BW, ROOS BA, DRAPER HH and LAMBERT PW, 1981. Vitamin D and its metabolites in human and bovine milk. *J Nutr*, 111, 1240–1248.

INSTITUT NATIONAL DE VEILLE SANITAIRE, 2004. Epidémie de Béribéri infantile à Mayotte. Rapport d'investigation, 59 pp. http://www.invs.sante.fr/publications/2004/beri_beri_071204/rapport_beriberi.pdf

JOHNSTON L, VAUGHAN L and FOX HM, 1981. Pantothenic acid content of human milk. *Am J Clin Nutr*, 34, 2205–2209.

LUCAS A, AGABRIEL C, MARTIN B, FERLAY A, VERDIER-METZ I, COULON JB and ROCK E, 2006. Relationships between the conditions of cow's milk production and the contents of

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

components of nutritional interest in raw milk farmhouse cheese. *Lait*, 86, 177–182.

LUTTER CK and RIVERA JA, 2003. Nutritional status of infants and young children and characteristics of their diets. *J Nutr*, 133, 2941S–2949S.

MARTIN B, FEDELE V, FERLAY A, GROLIER P, ROCK E, GRUFFAT D and CHILLIARD Y, 2004. Effects of grass-based diets on the content of micronutrients and fatty acids in bovine and caprine dairy products. In: Lüscher A, Jeangros B, Kessler W, Huguenin O, Lobsiger M, Millar N and Suter D (eds), *Land Use Systems in Grassland Dominated Regions*. Vdf, Zürich, 9, 876–886.

MCDERMOTT CM, BEITZ DC, LITTLEDIKE ET and HORST RL, 1985. Effects of dietary vitamin D3 on concentrations of vitamin D and its metabolites in blood plasma and milk of dairy cows. *J Dairy Sci*, 68, 1959–1967.

NATIONAL RESEARCH COUNCIL, 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. edn. National Academy Press, Washington, DC.

NOZIÈRE P, GRAULET B, LUCAS A, MARTIN B, GROLIER P and DOREAU M, 2006. Carotenoids for ruminants: from forages to dairy products. *Anim Feed Sci Technol*, 131, 418–450.

OKANO T, SHIMOMURA Y, YAMANE M, SUHARA Y, KAMAO M, SUGIURA M and NAKAGAWA K, 2008. Conversion of phylloquinone (vitamin K1) into menaquinone-4 (vitamin K2) in mice. Two possible routes for menaquinone-4 accumulation in cerebra of mice. *J Biol Chem*, 283, 11270–11279.

POWERS HJ, 2003. Riboflavin (vitamin B2) and health. *Am J Clin Nutr*, 77, 1352–1360.

REEVE LE, JORGENSEN NA and DELUCA HF, 1982. Vitamin D compounds in cow's milk. *J Nutr*, 112, 667–672.

ROJE S, 2007. Vitamin B biosynthesis in plants. *Phytochem*, 68, 1904–1921.

SANTSCHI DE, BERTHIAUME R, MATTE JJ, MUSTAFA AF and GIRARD CL, 2005. Fate of supplementary B-vitamins in the gastrointestinal tract of dairy cows. *J Dairy Sci*, 88, 2043–2054.

SCHWAB EC, SCHWAB CG, SHAVER RD, GIRARD CL, PUTNAM DE and WHITEHOUSE NL, 2006. Dietary forage and nonfiber carbohydrate contents influence B-vitamin intake, duodenal flow, and apparent ruminal synthesis in lactating dairy cows. *J Dairy Sci*, 89, 174–187.

SHEA MK and BOOTH SL, 2008. Update on the role of vitamin K in skeletal health. *Nutr Rev*, 66, 549–557.

SHEARER MJ and BOLTON-SMITH C, 2000. The UK food data-base for vitamin K and why we need it. *Food Chem*, 68, 213–218.

SHEARER MJ, BACH A and KOHLMEIER M, 1996. Chemistry, nutritional sources, tissue distribution and metabolism of vitamin K with special reference to bone health. *J Nutr*, 126, 1181S–1186S.

SMILOWITZ JT, DILLARD CJ and GERMAN JB, 2005. Milk beyond essential nutrients: the metabolic food. *Austr J Dairy Technol*, 60, 77–83.

STABLER SP, LINDENBAUM J and ALLEN RH, 1997. Vitamin B-12 deficiency in the elderly: current dilemnas. *Am J Clin Nutr*, 66, 741–749.

SUHARA Y, WADA A and OKANO T, 2009. Elucidation of the mechanism producing menaquinone-4 in osteoblastic cells. *Bioorg Medic Chem Lett*, 19, 1054–1057.

USDA NATIONAL NUTRIENT DATABASE FOR STANDARD REFERENCE, 2009. NDB No. 01077, Milk, whole, 3.25% milk fat. http://www.nal.usda.gov/fnic/foodcomp/Data/ (15 April 2009).

USUI Y, TANIMURA H, NISHIMURA N, KOBAYASHI N, OKANOUE T and OZAWA K, 1990. Vitamin

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

K concentrations in the plasma and liver of surgical patients. *Am J Clin Nutr*, 51, 846–852.

VAN WINCKEL M, DE BRUYNE R, VAN DE VELDE S and VAN BIERVLIET S, 2009. Vitamin K, an update for the paediatrician. *Eur J Pediatric*, 168, 127–134.

VANDERSLICE JT, BROWNLEE SG, MAIRE CE, REYNOLDS RD and POLANSKY M, 1983. Forms of vitamin $B_6$ in human milk. *Am J Clin Nutr*, 37, 867–871.

WEAVER CM and FLEET JC, 2004. Vitamin D requirements: current and future. *Am J Clin Nutr*, 80 (suppl.), 1735S–1739S.

WEISS WP, 2001. Effect of dietary vitamin C on concentrations of ascorbic acid in plasma and milk. *J Dairy Sci*, 84, 2303–2307.

WEISS WP, HOGAN JS and SMITH KL, 2004. Changes in vitamin C concentrations in plasma and milk from dairy cows after an intramammary infusion of *Escherichia coli*. *J Dairy Sci*, 87, 32–37.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

# 10

# Managing the environmental impact of the dairy industry: the business case for sustainability

**R. Pagan, N. Price and P. Prasad, The University of Queensland, Australia**

IP Address: 129.132.208.100

**Abstract:** Increasingly stringent workplace, food safety and environmental regulations, growing customer expectations, limited resources and climate change mitigation measures are just some of a growing list of pressures currently facing the dairy industry. In order to remain competitive it is becoming increasingly accepted that the entire supply chain needs to explore opportunities to manage and improve resource efficiency and to reduce waste. This chapter looks at eco-efficiency initiatives undertaken on dairy farms and processing sites that have achieved both environmental and economic gains. The authors draw on case studies from around the globe and their own studies in Australia to demonstrate that eco-efficiency really does make good business sense and is an essential component of the industry's long term environmental and financial sustainability.

**Key words:** eco-efficiency, cleaner production, waste management, waste minimisation, water efficiency, water minimisation, energy management, environmental improvement, dairy waste management.

## 10.1 Environmental challenges facing the dairy industry

One of the biggest challenges facing food producers and processors today is managing and reducing environmental impacts while also staying viable in an increasingly competitive marketplace. While compliance and environmental legislation have played an important role in setting requirements for managing

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

environmental emissions, the dairy industry is now actively being encouraged to play a more proactive role in improving its environmental performance. This has included, for example, industry codes such as those for dairy farm effluent management and the development and certification of environmental management systems.

Effective resource management has become critical with pressure worldwide on fresh water supplies and a dependence on non-renewable fossil fuels. In recent years many countries have introduced water and energy efficiency programmes and greenhouse abatement initiatives to encourage more sustainable use of these limited resources. The Kyoto protocol and emergence of emissions trading schemes have provided opportunities for industry to trade carbon permits and invest in green(er) technologies.

In some countries, full-cost recovery regulations for water supply and wastewater treatment have also encouraged not only water efficiency but also product and chemical recovery leading to higher product yields and improved effluent quality. The impact of solid waste disposal has also become a significant environmental issue for the dairy industry, with many manufacturers signing packaging agreements and looking at the environmental impacts of packaging from production through to disposal by the customer. This chapter looks at the environmental impacts of the dairy industry from the paddock to the manufacturer's gate and demonstrates through various case studies how innovative farmers and manufacturers are endeavouring to sustainably manage environmental impacts by adopting the principles of eco-efficiency.

Eco-efficiency is about systematically evaluating existing practices to identify opportunities to minimise resource consumption or waste production. Eco-efficiency not only benefits the environment but is also a 'win-win' business strategy. Cost savings can be made directly from reduced raw material, water and energy usage. Not only is waste a loss of valuable material but its storage, handling, transport, treatment and disposal are also costly. Improved efficiency also often means reduced maintenance and operational costs. There may also be many indirect benefits that are important in today's marketplace such as reduced exposure to risk and liability, improved relations with regulators with the focus being on self-regulation, and even a safer work environment as managers explore less hazardous, environmentally friendly alternatives. In some cases there can also be opportunities for a competitive advantage through product diversification or greater ability to attract investors or customers through promotion of a 'green' image. Thus eco-efficiency demonstrates the 'business case' for better environmental management practices.

IP Address: 129.132.208.100

## 10.2 The environmental impacts of dairy farming

### 10.2.1 Changing soil characteristics

Possibly the first step towards more sustainable milk production involves becoming more aware of the land and its characteristics. Sustainable farming

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

means having and using soils and other inputs sustainably. As with many eco-efficiency opportunities the best results are frequently achieved by implementing simple, commonsense and often low-cost measures. This is particularly true when it comes to preventing soil compaction, erosion, poor drainage, soil acidity and nutrient deficiencies on dairy farms. Using monitoring and mapping techniques to identify the major soil types, rivers, streams, dams and drainage on the site (including wet soils and other drainage systems) is an effective method to highlight areas requiring varying farm management regimes.

*Soil compaction*

Soil compaction by cattle hooves and cultivation practices can destroy the soil structure and restrict the movement of air, water and roots through the soil. When looking at eco-efficiency opportunities it is always better to reduce or eliminate the cause of the impact. Eldridge (2004) suggests a number of good farming practices to prevent or fix soil compaction:

- Minimising tillage, e.g. sowing pastures using direct drilling (deep ripping of paddocks may be required if the soil is already compacted)
- Slashing and mulching pasture to add organic material to the soil rather than burning it
- Growing deep-rooted pastures to help break up compacted soil
- Cultivating the soil only when the soil moisture content is suitable
- Using fences to separate wetter areas from drier locations so stock and vehicles can be kept off these areas during wet periods
- Installing feeding pads and breaking up the herd by installing a number of watering and shade areas
- Confining cattle traffic to established and raised laneways (Eldridge, 2004).

IP Address: 129.132.208.100

**Case Study 1**

A dairy farm in New South Wales, Australia, established a network of gravel laneways, gully crossings and a bridge to reduce erosion and soil compaction and to improve access during wet periods. Watering troughs were also located in all paddocks to reduce the impact of stock on the riparian zones. Apart from the many environmental benefits including less pugging (hoof depressions) and erosion, the redirection of nutrients in the runoff from the laneways into the paddocks and improved river water quality, the infrastructure has allowed the farm to increase its herd size from 130 to 300 head. Table 10.1 shows the direct financial savings resulting from this work (NSW Department of Environment and Conservation, 2004).

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

**Table 10.1** Direct financial savings (AUS$) from soil conservation work

| Works implemented | One-off costs | Areas where savings were made | Estimated savings (AUS$) |
|---|---|---|---|
| *Laneways* | | Reduced labour | 4,200 |
| Gravel for the lanes | 75,000 | Tractor savings | 2,200 |
| Laneway contractor | 75,000 | Motorbike savings | 5,500 |
| Bridge | 25,000 | Reduced mastitis (10%) | 300 |
| Culverts | 10,000 | Nutrient recovery | 1,800 |
| *Stock watering system* | | Reduced land width | 3,000 |
| Pipework and tank | 24,000 | **Total savings per year** | **17,000** |
| Pumps | 3,000 | **Productivity gain per year** | **75,000** |
| Troughs | 18,000 | **Total cost-benefit per year** | **92,000** |
| **Total one-off costs** (includes estimates to complete the work) | **230,000** | **Payback period: 3–5 years** | |

Source: NSW Department of Environment and Conservation (2004).

*Soil and river bank erosion*

During the last 40 years about 30% of the world's arable land has become unproductive with around 75 billion tons of soil being lost every year worldwide. As it takes on average around 500 years to replace 25 mm of soil, erosion has now become a huge global concern (Pimentel, 2006). Again, efficient management practices can help prevent the loss of this vital resource.

Rotational grazing, where livestock are moved between paddocks, can help to maintain healthy pasture cover essential for soil health. This good farming practice requires subdividing the land into paddocks, providing access to water, and constant monitoring, including grazing durations. The US National Sustainable Agriculture Information Service has produced a guide with further resources to assist farmers interested in implementing a rotational grazing system (National Sustainable Agriculture Information Service, 2004). Decision support software is also available to help producers determine stocking strategies or even to test the benefits of rotational grazing before making capital investments. For example, the Australian software 'PaddockGRASP' simulates pasture growth within individual paddocks up to 12 months in advance. The model, of course, requires the input of information such as soil types, tree cover, terrain and pasture cover as well as historical climate data and any existing land degradation issues (MCV, 2007). Case study 2 illustrates financial benefits in adopting a rotational grazing scheme for a US farm.

Riverbanks are an area of prime environmental concern as erosion is not only dangerous for stock but results in a loss of valuable nutrients and

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

**Case Study 2**
The Scherping family managed a 200-acre dairy farm in Minnesota for 15 years by ploughing soils and growing corn crops that required both fertilisers and herbicides. By 2000 the farm had totally moved away from planting up cornfields to instead utilising permanent pastures, which were grazed on a rotational basis. In 2000 the farm was compared with 564 other dairy farms in Minnesota. The expenses were 50% of gross income compared to 'confined corn fed' dairy farms where higher production costs were about 75% of gross income. Table 10.2 shows the financial savings made (RTC, 2005).

**Table 10.2** Financial savings (US$) from rotational grazing

| Farm | Gross income | Direct costs | Overhead costs | Annual profit |
|---|---|---|---|---|
| Scherping dairy | 125,000 | 54,000 | 8,000 | 63,000 |
| Confinement farms | 180,000 | 107,000 | 9,000 | 54,000 |

Source: RTC (2005).

farmland. Once unstable, the river bank is more susceptible to flood damage and noxious weeds, Key management strategies to prevent or repair riparian erosion include fencing waterways to exclude or restrict stock, maintaining riparian buffer zones, building dedicated stock crossings, locating watering points and shelter away from waterways and replanting native vegetation on eroded waterways. Often the placement of water points away from the waterways has helped producers better utilise the paddocks (Jansen and Robertson, 2001).

IP Address: 129.132.208.100

*Loss of soil fertility*
It is important that nutrients leaving the farm in the form of milk are replaced. Computer software is available to producers, such as 'Dairybal', which is a whole farm nutrient and water mass balance spreadsheet (Queensland Department of Primary Industries and Fisheries, 2008). The program can calculate the waste output from a dairy herd according to the rations they are fed and types of pastures or crops on which they graze. It then apportions the waste between the milking shed, the yards, the feed pad and up to six paddocks. Such software can determine whether the effect of current or proposed cropping or pasture management practices on nutrient loading is sustainable. It also identifies areas where inorganic fertilisers may be required, thereby avoiding the detrimental environmental impacts of over-fertilising. Case study 3 demonstrates how a 210-cow farm in New Zealand reduced its fertiliser use by conducting a nutrient balance.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

**Case Study 3**
Louis and Barbara Kuriger from the North Island of New Zealand undertook a nutrient budget. Based on 2002 fertiliser application rates, they were able to reduce their phosphate rate from 71 kg P/ha/yr to 48 kg/ha/yr, saving around NZ$5000 in phosphate application alone. The impact of reducing annual fertiliser application was analysed in 2004 against previous soil tests and showed that the soil phosphate level had remained constant despite constant farm production (Dairy New Zealand, 2005).

Along with the benefits discussed previously, rotational grazing can also help to ensure that manure and urine are returned to where the feed was initially grazed. Also around 8–10% of a dairy cow's manure and urine can be recovered from the milking yards (Hopkins, 2004). If well managed, this effluent can be a valuable resource that can improve pasture production and reduce fertiliser use. Various methods are available, including direct return to fields and composting.

**Case Study 4**
Rosenholm Farm in Wisconsin has used the organic solids remaining after dairy manure has been flushed, together with bedding material, to produce compost for the commercial market for over 14 years. The dairy spends about 10% of what it would take to haul and land-apply all their manure. Only lagoon liquids are used on farm (National Dairy Environmental Stewardship Council, 2005).

IP Address: 129.132.208.100

A US study on the feasibility of composting for small to medium-sized farms suggests it can be economically viable if farmers use existing equipment, rented machinery and hired help or are part of a cooperative arrangement between a number of farms (Center for Integrated Agricultural Systems, 1996). The article provides cost comparisons between manure handling methods. Organic solids separated from the manure can even be utilised as bedding with some systems (Johnson, 2007).

Returning dairy effluent to paddocks or pastures is an effective way to help restore the nutrient balance. As much as 90% of the nutrient value (nitrogen, phosphorus, potassium, sulphur and trace elements) in the effluent can be retained in a well-designed system and management plan (Dairy Insight *et al.*, 2007). Tables 10.3 and 10.4 give an indication of the value of effluent for two feeding schemes. However, as in all intensive practices, care must be taken not to overload the paddocks with nutrients or to create nuisances.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

**Table 10.3** Dollar value (NZ$) of effluent from 100 cows on an all grass system

| Nutrient | Solid fertiliser equivalent | Dollar value |
|---|---|---|
| N | 13 t urea | 650 |
| P | 0.7 t superphosphate | 130 |
| K | 1.1 t MoP | 450 |
| Mg | 0.2 t MgO | 70 |
| Total dollar value | | 1300 |

Source: Dairy Insight *et al.* (2007).

**Table 10.4** Dollar value (NZ$) of effluent from 100 cows fed maize on a feed pad

| Nutrient | Solid fertiliser equivalent | Dollar value |
|---|---|---|
| N | 2.3 t urea | 1080 |
| P | 1.3 t superphosphate | 250 |
| K | 2.1 t MoP | 830 |
| Mg | 0.3 t MgO | 100 |
| Total dollar value | | 2300 |

Source: Dairy Insight *et al.* (2007).

### 10.2.2 Water contamination and consumption

IP Address: 129.132.208.100

Besides the good farming practices outlined above, there are other opportunities to reduce the impact of effluent generated on dairy farms. Reducing the volume of water used in the dairy and yards will reduce the amount of effluent generated. The amount of water used per cow can vary considerably. An Australian study, for example, showed that in the south-west of Victoria the most efficient dairies used less than 2000 L per cow annually, while the least efficient used 38,000 L per cow (Victorian Environmental Protection Agency, 2007). Dairy Australia (2008) suggests how producers can improve water efficiency and effluent quantity and quality through:

- sweeping or scraping floors and yards before washing to reduce the solid content in the effluent;
- periodic damping of yards and the use of scrapers (including gate scrapers with blade or chain), which can reduce the amount of water needed for final cleaning;
- design of yards to reduce cleaning requirements e.g. good slope and drainage and weeping walls or solid separators to remove solids;
- using non-stick coatings for dairy walls and floors;
- allowing the cows a few minutes in the paddocks in the early morning so manure falls in the paddock before moving them to the shed;
- installing a barrier foot bath or using sawdust to reduce the amount of material brought into the yards to reduce cleaning requirements;

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

- harvesting of rainwater to supplement water supplies that can assist in effluent digestion;
- recycling effluent from a multiple pond system for washdown;
- using harvested rainwater for plate coolers and then storing and reusing cooling water for yard washing or return to stormwater dams;
- using recycled effluent or plate cooler water for flood washes as this method requires large volumes of water;
- correctly sizing plate coolers and interlocking water and milk pumps so water is going to the plate cooler only when milk is flowing (water flow can be further optimised by installing a variable-speed drive on the pump on more sophisticated systems);
- dry cleaning and considering reducing the size of hoses;
- checking and repairing nozzles and hoses regularly;
- making sure hoses have trigger nozzles and are not excessively long;
- turning off cup and platform sprays in rotary sheds after milking;
- making sure milking machines capture final rinse water for the initial rinse and that the volumes of water used for rinsing are not excessive;
- recycling wash water to recover not only water but also detergents;
- considering air injection on older milk lines, which can reduce water used for pipe cleaning by 20–30%;
- checking vat cleaning matches manufacturer's requirements and ensuring spray heads are checked and cleaned regularly;
- for larger farms, considering the dietary intake of cows. Adjusting feed without compromising stock health can have an impact on phosphate and nitrogen levels in the effluent stream (McDonald, 2006);
- not allowing clean stormwater to mix with effluent.

IP Address: 129.132.208.100

The UK Milk Development Council has produced an excellent guide called 'Effective Use of Water on Dairy Farms' that can assist farmers to evaluate how efficiently they are using water on their farms and that includes three detailed farm audits (MDC, 2007).

Fertiliser should be applied under the optimum conditions (i.e. not when heavy rain is expected or when water tables are very high) and when plant uptake is greatest. Soil and effluent testing can help to ensure that nutrient applications are not in excess of the plants requirements and best practice application methods to protect riverbanks and streams should be used (Watschke, 1998).

Excluding stock from riverbanks and saturated soils as discussed previously is not only essential to prevent erosion but also avoids nitrogen from urine and phosphorus from manure that can cause algae growth. Riparian vegetation not only takes up nutrients for plant growth but also filters surface runoff and denitrifies groundwater (Hoorman and Cutcheon, 2005).

### 10.2.3 Air emissions and energy consumption

Greenhouse gases absorb the sun's heat and make the atmosphere warmer. The three main greenhouse gases released during dairy farming and production are

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

carbon dioxide ($CO_2$), nitrous oxide ($N_2O$) and methane ($CH_4$). Nitrous oxide is characterised as having 296 times more global warming potential than carbon dioxide, and methane as having 23 times more global warming potential than carbon dioxide (Energy Information Administration, 2003). All three greenhouse gases are produced during combustion of fossil fuels in production of electricity; however, there is significantly more impact due to enteric emissions of methane, i.e. that which is produced by microbes in the cow's rumen during digestion. Around 80% of the methane emissions come from enteric fermentation and 20% from manure management (Vergé *et al.*, 2007). Nitrous oxide is also produced by microbes in waterlogged soils converting nitrogen from cow urine, fertiliser or legumes into nitrous oxide. Producers can calculate methane, nitrous oxide and carbon dioxide emissions using tools developed in Australia: 'GrassGro' and 'DairyMod' (Victorian Department of Primary Industries and University of Melbourne, 2008).

Methane emissions can be reduced by running fewer cows and managing them more productively through improved genetics and nutrition to achieve the same yields. Matching animal feed to efficiency (milk yield) makes good business sense, reduces waste and optimises outputs. The Queensland dairy industry, for example, was able to reduce methane release by 6% between 1988 and 1996 by dropping cow numbers while improving milk production from 2924 L per cow in 1988 to 4046 L per cow in 1996 (Davidson, 2000).

Research is being undertaken to find additional technologies to reduce ruminant methane production. These include the following:

IP Address: 129.132.208.100

- Addition of unsaturated fatty acids such as coconut, linseed, canola and cod liver that act as a sink for hydrogen (New Zealand Ministry of Agriculture and Forestry, 2003).
- Antibiotics such as monensin, which encourages growth of *Streptomyces* spp. The National Research Council (2001) found increases in milk production, better feed conversion efficiency and reduced acidosis, ketosis and bloat; however, it appears the effects decrease with repeated use (O'Mara, 2004; Davidson, 2000).
- Organic acids such as malate, fumarate, citrate and succinate that will use the hydrogen gas, thereby starving the methane microbes. While they are effective they may be costly as a feed supplement (O'Mara, 2004). Breeding high malate or fumarate forages may be an alternative (Eckard and Hegarty, 2004).
- Bacteria called acetogens that convert carbon dioxide and hydrogen to acetate rather than methane. These acetogens occur naturally in ruminants, humans and wood-eating termites but their activity needs to be promoted (Davidson, 2000).
- A vaccine against methanogens is being investigated by the CSIRO in Australia.
- Secondary metabolites used for the manipulation of rumen fermentation by selective inhibition of a microbial group, e.g. ethanol extract of soapnut seed pulp administered during regular milking to remove protozoa (Kamra *et al.*, 2006).

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

Nitrous oxide emissions can be reduced by not applying nitrogen fertiliser at high application rates or before heavy rainfall is predicted. To ensure maximum uptake, nitrogen fertiliser should also only be applied when the pasture is actively growing. Producers should avoid applying fertiliser or overstocking paddocks with waterlogged soils. Recent research trials by the University of Melbourne in Australia for the Pastoral Greenhouse Gas Research Consortium show that nitrification inhibitors (substances that slow or stop the conversion of soil ammonium to nitrate) have the potential to reduce nitrogen losses by up to 60–70% (PGGRC, 2006).

Producers can also reduce carbon dioxide emissions and energy costs by reducing electricity use and investigating renewable energy options. Electricity usage on dairy farms can range between 200 kWh and 400 kWh per cow per annum (MDC, 2005).

Heating water accounts for around 40% of electricity use on farms (Dairy SA, 2008a). As discussed previously, reducing water consumption for cleaning is the best way to reduce heating requirements. Other opportunities include using other options to pre-warm the water. A reduction in greenhouse gases of 139 g per 100 L of water is possible for every 1ºC increase in water temperature that can be achieved before it enters the electric hot water service (Dairy SA, 2008a). Preheating options include solar heating, heat recovery from the refrigeration unit, heat capture from cleaning wastewater or the heat in the milk itself as it passes through the plate cooler, a heat pump, or even alternatives such as geothermal sources.

IP Address: 129.132.208.100

**Case Study 5**

The O'Regan family manages 630 cows on their Awarua Farm at Tahuna, New Zealand. The farm's milking machine was allowing relatively hot cleaning water to go to waste. By installing a heat exchanger to recover heat from wastewater to preheat incoming water, the farm achieved an annual energy saving of 30% (2245 kWh) or a reduction in $CO_2$ emissions of about 1 tonne per year (EECA, 2007).

When looking at the actual electric hot water system, producers should ensure the water is heated only when it is needed, that the storage tank and pipes are insulated and that the thermostat is set correctly, i.e. is not overheating the water. A study by the Farm Energy Centre showed an uninsulated tank will lose about 50% of its energy over a 17-hour period while only 5% will be lost from a tank that is well insulated (MDC, 2005).

Cooling milk on average uses around 30% of the dairy farm's total energy consumption. A reduction in greenhouse gases of 37.5 g per 100 L of milk is possible for every 1ºC decrease in milk temperature that can be achieved before the milk enters the vat (Dairy SA, 2008b).

© Woodhead Publishing Limited, 2010

Downloaded From Vet-eBooks.com

Energy savings may also be possible by improving the efficiency of vacuum and milk pumps. Opportunities include installing variable-speed drives that adjust pump speed according to the demand rather than having the pump operating at a constant speed. Water pumps and pipes should be correctly sized for the load with no unnecessary bends or elbows. Energy-efficient new electric motors should replace old ones, including turn-off standby features when the cluster is not milking. Other opportunities include installing energy-efficient equipment and lighting, which should be task specific (e.g. general lighting for laneways and holding yards and specific lighting for office and veterinary areas) and maximum use should be made of natural lighting through the use of skylights.

### 10.2.4 Farm wastes and chemicals

Dairy farms produce solid non-biodegradable wastes such as containers, packaging, scrap metal and drums along with plastic silage wrap. Biodegradable wastes include milk and deceased stock. In many cases there will also be waste chemicals or chemical residues. The waste hierarchy should be considered when dealing with waste, i.e. first eliminate, then reuse or recycle, recover, and lastly dispose as the least preferred option. Disposal of all waste must be in accordance with relevant legislation or advice from relevant local authorities.

Waste milk can be fed to animals if the farm has suitable storage facilities (up to 10 L per day per cow). A less preferable option is to apply it to the land away from watercourses at a rate of 1:10 milk to water (only during dry periods) or to dispose milk to effluent ponds (only possible for one or two days) (Dairy Australia, 2003).

Poor management of deadstock can lead to disease, odour and the contamination of groundwater. Carcasses can be composted if a dedicated area is set aside and the producer has a sound knowledge of the composting process, otherwise it is preferable to have them removed by a knackery. Deep burial in areas with a low water table or burning are the least preferable options (Dairy Australia, 2003).

Solid non-biodegradable wastes such as food wastes and green wastes can be composted and materials such as timber and wire reused. Lead batteries, scrap metal and oil (sump, engine, gear and hydraulic oil) can be recycled and plastic containers and drums returned if possible to the supplier or a recycler collection point. In some areas recycling is an option for silage wrap and baling twine (Central Murray Regional Waste Management Group, 2008). Recycling can significantly reduce the cost of sending material to landfill.

Solid waste will be reduced through the use of best practices in the design and operation of the dairy process, from field to parlour, including applied eco-efficiency, optimisation of yield, benchmarking, modern milking and dairying practices, and herd management, including selection of calving times for optimum yield and feed efficiency.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

### 10.2.5 Loss of habitat and biodiversity

Biodiversity is the natural variety of native wildlife together with the environmental conditions necessary for their survival. It is acknowledged that most dairy farms use land that has been extensively farmed for many years; however, it is possible for the dairy farmer to manage for biodiversity and enhance the natural habitat as well as improving the farmed land. A recent study of dairy farmers in South-west Victoria, Australia, examined a range of biodiversity issues. Eighty percent of farmers in the study were looking for practices which provided them with a productivity return and were unlikely to use specialist environmental indicators to monitor resource states. They would, however, use information that has both a production and an environmental benefit such as soil tests. Thirty percent of farmers wanted indicators that can help them to benchmark their environmental performance against farmers similar to themselves. Twenty percent of the farmers were thinking of the next generation and placed a high value on biodiversity practices (Parminter and Nelson, 2003).

Dairy farmers can help in biodiversity conservation in many ways, such as by improving farm management practices by, for example, conserving and restoring native vegetation and providing protected areas, managing feral animals, improving vegetation levels, using conservation methods and best-practice grazing and tilling, restoring eroded or saline soils and managing water on site effectively.

IP Address: 129.132.208.100

## 10.3 The environmental impacts of dairy processing

### 10.3.1 Consumption of non-renewable fuel and greenhouse gas emissions

The transport of milk from the farm to the processing plant and from the plant to distribution outlets consumes energy (usually non-renewable fuels) and releases greenhouse gas emissions. Global trade also means more milk products are travelling even further distances. The transport sector accounts for 15% of global carbon dioxide emissions and 31% of ozone released into the atmosphere (Butler, 2008). Tim Lang developed the concept of 'food miles', which looks at the ecological as well as social and economic consequences of food production to help consumers make judgements about which products they purchase (Woodhouse, 2007). Food miles, however, can be misleading and a lifecycle approach is a better tool for consumers to evaluate food systems as it includes production as well as transport and distribution. For example, a report produced by Lincoln University in New Zealand compared the efficiency of dairy production in Europe and New Zealand. The findings claim that because New Zealand animals graze on grass all year round rather than eating feed concentrates, they are twice as energy efficient, even with export transport costs included (Woodhouse, 2007).

An Australian life cycle study by Lundie *et al.* (2003) indicated that around 49% of total energy use is associated with milk manufacturing, 43% with

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

production on-farm and 8% for milk transport. In processing, energy is consumed for refrigeration, the operation of machinery and general lighting and air-conditioning (cooling and heating). Typically the main sources for this energy are non-renewable fossil fuels such as natural gas, coal, oil, LPG (liquid petroleum gas) and electricity.

*Energy management*

Good energy management is an important key in identifying the end uses of energy and opportunities for improvement. This may involve the formation of an energy team and the installation of steam, gas and electricity sub-meters to track energy use across the site.

*Evaporators and driers*

A wide range of energy efficiency opportunities exists through optimising the operation of energy-consuming equipment. For example, evaporators that are used to concentrate milk can be single or multiple-stage (effect). By increasing the number of effects, significant efficiencies can be obtained. Some large factories in Europe have been able to increase the number of effects up to seven (ETSU, 1998). In some cases evaporators may be replaced with membrane units where the equivalent level of concentration can be attained depending on viscosity, etc.

**Case Study 6**

A whey processing plant in the Netherlands uses a nanofiltration unit to concentrate and desalinate whey instead of an evaporator and ion exchange unit. The unit increases the solid content from 5.5% to 17% and removes 70% of the salt content from the permeate. Overall energy consumption has decreased by 70% of the original consumption, largely due to a reduction in steam production, in addition to water and chemical savings from reduced cleaning requirements. The payback period was 1.3 years (CADDET, 1999).

IP Address: 129.132.208.100

Usually used in conjunction with evaporators, spray driers deliver milk droplets into a chamber of hot air to dry product to 50–90% solids content. Considerable savings can be made by maximising the solids content of the milk input. For every 0.5% increase in milk solids, energy consumption is reduced by 2% (ETSU, 1996). Energy may be recovered from drier exhaust streams.

*Boilers*

The efficient operation of boilers will reduce both energy and water consumption. Having optimum ratios in the boiler of air to fuel will ensure the optimum mix of flue gases. Insufficient air causes incomplete combustion while excess air leads to heat loss in the boiler stack. Installing an oxygen trim control on boilers can lead to considerable fuel savings, as shown in Table 10.5.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

**Table 10.5** Fuel savings from installing online oxygen trim control

| Boiler capacity (MW) | Fuel savings (GJ/yr) | Fuel savings (AUS$/yr) | $CO_2$ emissions (t/yr) | Payback period (yr) |
|---|---|---|---|---|
| 0.5 | 318 | 3,816 | 19 | 2 |
| 1 | 635 | 7,620 | 37 | 1 |
| 2 | 1,270 | 15,240 | 75 | 0.5 |
| 4 | 2,540 | 30,480 | 150 | 0.2 |
| 6 | 3,810 | 45,720 | 224 | 0.2 |
| 8 | 5,080 | 60,960 | 299 | 0.1 |
| 10 | 6,350 | 76,200 | 374 | 0.1 |

Assumptions: gas costs AUS$12/GJ; boilers operating 24 h/day, 350 days/year; installation cost of the boiler.
Source: SEAV (2004).

**Table 10.6** Effects of soot and scale on heat transfer

| Build-up (mm) | Heat loss (%) | Increased fuel consumption (%) |
|---|---|---|
| *Soot* | | |
| 0.8 | 12 | 2.5 |
| 1.6 | 24 | 4.5 |
| 3.2 | 47 | 8.5 |
| *Scale* | | |
| 0.8 | 8 | 2 |
| 1.6 | 12 | 2.5 |
| 3.2 | 20 | 4 |

Source: Spielmann (n.d.).

IP Address: 129.132.208.100

Other typical boiler efficiency measures include monitoring the stack gas temperature regularly. A major variation is an indicator that either the boiler tubes need to be cleaned or the air-to-fuel ratios need to be adjusted. The accumulated soot or scale acts as an insulator and can inhibit effective heat transfer, as shown in Table 10.6.

Boilers should be operated at design working pressure. If lower pressures are required, pressure-reducing valves should be employed. The boilers in many dairy processing plants have a steam supply potential far in excess of the site's actual needs, to cater for short peaks in demand. Good communication between the boiler operators and the end users can help reduce fuel wastage.

It is important to ensure minimal heat losses from the system. Opportunities include recovering boiler condensate and preventing the loss of live steam by repairing leaks and faulty steam traps and promptly replacing damaged insulation. For example, failure to repair 1 $m^2$ of un-insulated surface carrying steam at 700 kPa will lose around 225 MJ in a 24-hour period. That is approximately 81,000 MJ of natural gas or 2 tonnes of fuel oil per year (SEAV, 2002).

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

**Case Study 7**
Murray Goulburn in Victoria, Australia, saved AUS$180,000 annually (1536 tonne reduction in greenhouse emissions) by improving coordination between the boiler house and the operations team. Before the initiative the boiler attendant was only aware of the need for steam about 40% of the time compared to 95% after steps were made to improve communication (Industry, Science and Resources, 2003).

Regular checking and maintenance of steam traps are important. If traps fail to close, uncondensed steam and heat will escape, while if a trap fails to open the system becomes waterlogged and system performance is reduced. The insulation of steam and condensate return lines can reduce heat loss by up to 90%, as Table 10.7 illustrates.

The layout of steam lines also affects heat losses. Where possible, rationalise the length and sizing of the steam pipework, avoid excessive pressure drops and ensure pipes are sloping to ensure condensate drains to steam traps. Boiler efficiency can be further improved by installing heat recovery equipment such as economisers and recuperators. Economisers are air-to-liquid heat exchangers that recover heat from flue gases to preheat boiler feed water, while a recuperator is an air-to-air heat exchanger that recovers heat from flue gases to preheat combustion air.

IP Address: 129.132.208.100

*Refrigeration*

Refrigeration can consume up to 20% of a dairy processing plant's total energy costs (ETSU, 1998). Dairy processors typically use the vapour compression cycle refrigeration system consisting of a fluid called a refrigerant (usually ammonia). Optimising the system can save considerable amounts of energy (and money) (EEBPP, 2000).

There are several ways to increase evaporating temperature (pressure) and decrease condenser temperature:

- Not setting the thermostat in cold rooms and freezers lower than necessary.
- Sizing the evaporator and compressor to match the refrigeration load. A small

**Table 10.7** Heat losses from insulated and uninsulated steam lines

| Level of insulation | Heat loss (MJ/m/h) | Steam loss (kg steam/m/h) | Equivalent fuel cost (gas) per 50 m pipe per year (AUS$) |
|---|---|---|---|
| Uninsulated | 2.83 | 1.0 | 3396 |
| Insulated with mineral fibre | 0.14 | 0.05 | 165 |

Assumptions: 125 mm steel pipe at 150ºC; natural gas costs AUS$0.012/MJ; boiler operating 8 hr/day, 250 days/year.
Source: Prasad *et al.* (2004).

© Woodhead Publishing Limited, 2010

Downloaded from Vet-eBooks.com

evaporator may mean a lower initial capital outlay; however, running costs may be greatly increased by the need for a larger compressor to cope with the greater load. A condenser that is too large, on the other hand, can actually cause sub-cooling where the refrigerant starts to vaporise.

- The evaporator should be kept clean and defrosted when necessary to prevent the build-up of ice on the coil affecting the heat transfer. Hot gas from the outlet of the compressor can be used to defrost freezers if controls are accurate. The defrost water can then be used elsewhere in the plant.
- Condensers should be cleaned regularly to promote efficient energy transfer, and air-cooled condensers should have unrestricted airflow.

Reducing the load on the compressor is fundamental as it usually consumes 80–100% of the system's total energy use. The efficiency of the system is measured by the coefficient of performance or COP. This is the ratio of cooling output (kilowatts) compared with energy input (kilowatts), thus the higher the COP, the more efficient the system. The Australian Dairy Processing Engineering Centre has software available called 'Coldsoft' for plant personnel to review and improve the performance of refrigeration systems (DPEC, 2003).

It is important that the compressor capacity matches the load, as operating at partial loads will cause the compressor to stop and start frequently. The use of multiple compressors with a sequencing or control capability to match the load may help. Also ensure that the suction lines into the compressor are insulated. Case study 8 demonstrates how an effective control system and the temperature of the suction air can affect compressor efficiency.

IP Address: 129.132.208.100

**Case Study 8**

A Nestlé ice cream plant in Victoria, Australia, uses about 13 GWh annually (or approximately AUS$960,000) for refrigeration. A study showed that the compressor was operating under no load and stopping frequently because the suction temperature was 9ºC above the design temperature due to incorrect valve selection. The condenser pressure was also being maintained at around 1000 kPa even over winter months. By upgrading the computer control system the site was able to improve the valve selection, enabling the suction temperature to drop to 3ºC. The compressor now operates at a higher load, which has minimised stopping. The study also suggested reducing the condenser pressure to 750 kPa. The project saved AUS$100,000 annually in electricity costs, and compressor start-ups have been reduced by 92%. There was also a 20% overall reduction in plant maintenance. The project cost AUS$59,000 and took four months to install (SEAV, 2002).

While many of these opportunities can be costly, there are also many simple good housekeeping practices that can reduce the load on refrigeration systems.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

For example, up to 10% of the power consumption in refrigeration plants occurs from heat ingress through doorways. Automatic self-closing doorways, swinging doors or plastic strips are possible options for entrances frequently left opened. Lights and fans can also add heat to the system. Consider sensors or timers on lights, appropriate low energy consumption lighting and variable-speed drives that can cycle off fans and refrigerant feed during low-load times.

There are examples of some dairy plants using absorption chillers, which cool using low-grade heat produced elsewhere in or near the plant, e.g. low-grade steam, incinerated garbage, hot water, exhaust gas or solar energy. While the COP of absorption refrigeration is low, less than 0.8 kW of refrigeration for 1 kW of energy for a single-effect system, they can be a viable option if a reliable waste heat source is available.

**Case Study 9**
Honeywell Farms is a milk processing plant in the USA that uses a lithium bromide absorption chiller to cool the liquid refrigerant of the main refrigeration system below its saturation point. The system uses waste heat from a compressor driven by a natural gas engine. The system reduces the load on the compressor by 8–10% and saves US$90,400 annually. The payback period was 3.8 years (CADDET, 1996).

IP Address: 129.132.208.100

*Compressed air*

Around 10% of a dairy processing plant's total energy use is for compressed air used for purposes such as cleaning and operating filling and packing machines (ETSU, 1998). Again, compressed air systems are typically only 10–20% efficient, losing most of the electricity input in the form of waste heat. Because they are so inefficient it is important to select the correct type of compressor for specific applications. If the compressor needs to operate at variable rates, consider installing a control sequencing system or multiple compressors. Air receivers are another option to consider if the compressed air system needs to cope with occasional spikes. Installing a variable-speed drive may also be ideal for compressors that are oversized or operate at variable loads.

Other opportunities to reduce energy consumption in compressed air systems include identifying and repairing leaks using ultrasonic detectors, and simple methods such as soapy water on pipework or shutting the plant down and listening. Table 10.8 illustrates the cost of compressed air leaks.

There are many technical options to optimise compressor systems and as energy costs continue to rise these opportunities become more valuable – leaks, pressure drops, too-high set points, isolated equipment with long lines, dirty filters, etc., can all be managed with good maintenance and attention to detail. An interesting, easy-to-apply saving is to ensure the inlet air into the compressor is as cool as possible so that less energy is required to compress it. It is estimated

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

**Table 10.8** Cost of compressed air leaks

| Equivalent hole diameter (sum of all leaks) | Quantity of air lost per single leak ($m^3$/yr) | Cost of single leak (AUS$) |
|---|---|---|
| Less than 1 mm | 12,724 | 153 |
| 1 to 3 mm | 64,415 | 773 |
| 3 to 5 mm | 235,267 | 2,823 |
| Greater than 5 mm | 623,476 | 7,482 |

Assumptions: 700 kPa system operating for 4000 h/yr, electricity cost of AUS$0.08/kWh.
Source: SEDA (2003).

that every 3ºC drop in inlet air temperature decreases electricity consumption by 1% (SEDA, 2003).

Heat recovery from compressors for both refrigeration systems and compressed air may be viable if a potential application exists close to the heat source. A heat exchanger that recovers heat from compressor lubricating oil can heat water up to 90ºC without adversely affecting compressor performance. For example, a 37 kW single-stage oil-injected rotary screw compressor unit attached to a heat recovery unit can produce 36 L/min of 73ºC hot water (Atlas Copco, 2003).

*Other opportunities*

Lesser opportunities to reduce energy consumption in dairy processing plants include lighting and air-conditioning optimisation. Simply turning off lighting when not needed and installing energy-efficient lighting can provide easy savings, together with task lighting, occupancy sensors and natural lighting.

Some dairy companies are now also considering other alternative sources of energy, for example biofuels such as methane gas from anaerobic digesters, or sludge from wastewater treatment processes or separators. Table 10.9 illustrates the savings made by one ice-cream plant in New South Wales, Australia, which utilised methane gas from a lagoon digester. If there are opportunities, a regional

IP Address: 129.132.208.100

**Table 10.9** Sample methane and energy yields from biogas digestion for an ice cream factory in New South Wales

| | Low rate digestion of effluent |
|---|---|
| Material available for digestion | 3060 kg COD/day |
| Organic load available | 0.34 kg COD/$m^3$/day |
| Methane conversion rate | 0.352 $m^3$/kg COD removed |
| Organic removal rate | 70% |
| Methane yield | 754 $m^3$ $CH_4$/day |
| Energy yield | 27,000 MJ/day |
| Equivalent natural gas savings | AUS$324/day @ AUS$12/GJ |

Source: UNEP (1999).

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

digester may be able to serve several dairies, or even a mix of high BOD effluent from different types of plants.

Other alternative energy sources include solar and wind energy. While large roof spaces on dairy plants lend themselves to solar, capital installation costs are still high, especially when many plants are already recovering heat from other process equipment. Wind energy has been utilised in some dairy processing plants, although noise and the effect on visual amenity can constrain use. Some dairy plants are opting to reduce greenhouse emissions by using accredited 'green power'.

### 10.3.2 Water use

While in the past water has been considered a relatively cheap and expendable resource, there is now increasing awareness and acknowledgement of the true value of water. From the dairy processor's perspective the cost of water should not be viewed solely on the basis of its purchase cost but should also consider supply treatment, heating and cooling costs, wastewater treatment, pumping costs, disposal costs, capital depreciation and maintenance costs, as well as availability and reliability. Some of these costs are likely to rise considerably with many local authorities moving towards full cost recovery to supply freshwater and treat wastewater.

Envirowise in the UK has collected data from an independent survey of water use in dairies during 2004/05 and 2005/06 to develop a Water Account database, which enables dairies to enter their water use and obtain an instant indication of their performance with similar dairies (Envirowise, 2007). Measuring and monitoring data and its surveillance are important activities to generate key performance indicators and keep track of performance over time. Again, staff awareness and involvement along with monitoring water use with meters is essential in reducing water use, as Case study 10 illustrates.

IP Address: 129.132.208.100

**Case Study 10**

Dairy Farmers in New South Wales, Australia, installed 27 water meters after joining the Sydney Water business partnership programme 'Every Drop Counts'. A water assessment identified over AUS$300,000 of water savings for initial costs of AUS$150,000 and ongoing costs of AUS$26,000. Initiatives included preventing cooling tower overflow, recirculating homogeniser water, crate wash water and DAF water, reducing water for cleaning, repairing leaks and reviewing truck washing practices (Prasad *et al.*, 2004).

As staff behaviour is particularly important in reducing water consumption, managers could consider forming a water management team and displaying staff efforts to meet targets on graphs. Staff should always be encouraged to put

© Woodhead Publishing Limited, 2010

Downloaded From Vet-eBooks.com

**Table 10.10** Cost of water loss from leaking equipment

| Equipment | Hourly loss (L) | Annual loss (kL) | Water cost (AUS$/yr) |
|---|---|---|---|
| Union/flange (1 drop per second) | 0.5 | 5 | 12 |
| Valve (0.1 L per minute) | 6 | 53 | 128 |
| Pump shaft seal (0–4 L per minute) | 0–240 | 0–2,100 | 0–5,103 |
| Ball valve (7–14 L per minute) | 420–840 | 3,680–7,360 | 8,942–17,885 |
| 1-inch hose (30–66 L/minute) | 1,800–4,000 | 15,770–34,690 | 38,321–84,297 |

Assumptions: purchase cost of water AUS$0.54/kL, total cost AUS$2.43 (including wastewater treatment AUS$0.75/kL, wastewater pumping AUS$0.05 and wastewater discharge AUS$1.09).
Source: Envirowise (2003).

forward water-saving ideas and it may be beneficial to consider incentive schemes to reward good performance. Reducing the demand for water can be achieved in a number of different areas in dairy processing plants. These include reducing water used for processing, for cleaning, for operating utilities and for ancillary use. There are also water recycling and reuse opportunities.

IP Address: 129.132.208.100

*Leaks and process control*

One first easy step is to identify and repair leaks. Seemingly small leaks can actually lose significant amounts of water, as Table 10.10 demonstrates. As well as repairing visible leaks, it is also a good idea to regularly check the main supply meters during periods of non-production (i.e. no-flow periods) to ensure the plant has no hidden water losses.

Automating processes using control devices can help to reduce human error and the associated production costs. For example, water sprays for washing and lubricating equipment are frequently left operating unnecessarily when a simple solenoid linking sprays with conveyors or equipment motors could shut off valves to stop or start the water supply. Similarly, level controls on tanks can prevent water (and product!) overflowing to drain. Another, often very simple water-efficient measure is to optimise the flow of water to equipment using a flow regulator.

**Case Study 11**

Dairy Farmers in Victoria, Australia, were able to save AUS$19,800 annually by optimising the flow rate to the homogeniser with an AUS$259 flow regulator valve (Prasad *et al.*, 2004).

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

*Cleaning*

As cleaning can consume up to 50–90% of a dairy processing plant's total water consumption (Envirowise, 1999), it is an important area to conserve water. Opportunities include dry cleaning the equipment before washing using aids such as brushes, scrapers, and vacuums. Brooms, squeegees and mops can be used on floor surfaces. Managers could consider scrubbers and vacuum cleaners that can clean large floor surfaces effectively.

Trigger nozzles should be attached to any hoses used for washing down equipment and plant. An unattended hose left for one hour each day can waste 470 kL annually (260 days × 30 L/min × 60 min/day). A high-pressure water cleaner (typically using 4–20 L/min) may be a more efficient way to wash down some floor areas and around the wastewater plant; however, due to the possibility of aerosol contamination, their use in processing areas may be limited. In some cases it may be possible to reduce the amount of cleaning by:

- designing a better process layout. For example, ensure pipework drains efficiently and contains minimal bends or dead legs. Floors and wall surfaces should also be durable and easy to clean and designed to promote runoff;
- efficient scheduling of product changeovers;
- using a pig, an inert flexible plug or pulse of air, that is propelled through pipework to remove product rather than water flushing.

Clean-in-place (CIP) systems are automatically operated cleaning systems that deliver a number of wash and rinse cycles to the internal surfaces of a closed system. One of the main advantages of a CIP system is that it can allow the reuse of chemicals and water. While CIP systems are usually more water-efficient than manual cleaning they can become inefficient if they are not reviewed regularly. For example, cleaning cycles can be lengthened by operators to help rectify product quality issues, in-line monitoring instrumentation can drift out of calibration, and spray balls and nozzles can become worn due to the corrosive nature of the cleaning fluids and high operating temperatures. Burst rinsing, in which equipment is cleaned by a series of water bursts rather than a continuous stream, is also worth considering, although its viability will depend on the viscosity of the product.

IP Address: 129.132.208.100

**Case Study 12**

Peters and Brown in Western Australia, Australia, have adopted burst rinsing in their ice-cream CIP system and saved 15 ML annually. The company trialled burst rinsing in cheese processing but found it added excessive time to the cleaning cycle (Prasad *et al.*, 2004).

*Boilers and cooling towers*

Considerable amounts of water can also be lost through the operation of utilities such as cooling towers and boilers. The blowdown from boilers and cooling

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

towers prevents the build-up of dissolved solids that can cause scale. Installing a conductivity probe enables blowdown to be initiated only when the water exceeds a set value rather than relying on less accurate techniques such as manual flushing or timers. Blowdown can also be a good source for heat recovery. Equipment items, such as pumps, may also require water for cooling and sealing. If the water is flowing to drain after a single use it may be possible to recover it for other uses or to recirculate the water, as Case study 13 demonstrates.

**Case Study 13**
Murray Goulburn in Victoria, Australia, installed a water recirculation system on a vacuum pump. The water is now cooled using an air-cooled radiator, saving over 1 ML annually (Prasad *et al.*, 2004).

*Recycling and reuse*
There are also opportunities to reduce water consumption through the recycle and reuse of water. Boiler condensate should be recovered as a matter of routine and this significantly improves the efficiency of a steam system. Condensate water from evaporators is now commonly recovered for reuse; however, some treatment is usually required, e.g. the addition of a disinfectant such as chlorine dioxide. There are also examples of some processors using reverse osmosis membranes to polish evaporator condensate water. Condensate recovery can also have the benefit of recovering heat. Dairy processing plants using membranes to recover product also often recycle the permeate back into the process.

IP Address: 129.132.208.100

**Case Study 14**
Murray Goulburn in Victoria, Australia, processes 800 kL of whey into whey and lactose powder using ultrafiltration, nanofiltration and reverse osmosis (RO). The permeate from the RO plant is recycled back into the plant, saving 70,000 kL annually (Prasad *et al.*, 2004).

### 10.3.3 Solid waste

Dairy processors produce a considerable amount of solid waste that if minimised will reduce not only environmental impact and risk but also collection, treatment and disposal costs. When looking at the true cost of waste it is important to remember that waste is also just lost product or raw materials, so processing costs should also be included. There may even be an increase in revenue if product can be successfully recovered or a market found for new co-products.

Typical dairy solid wastes include non-organic wastes such as cardboard

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

boxes, plastic wrap, bottles and caps, foil seals, liquid board, labels, plastic and metal containers and drums, office waste and many others. Processors also produce a significant amount of organic waste including rejected product, returned final product, raw materials, out-of-date materials, lab samples, separator de-sludge, baghouse fines, effluent sludge and fat recovered from effluent. In Australia the dairy sector on average generates 9 kg of solid waste for every tonne of finished product, with almost 86% being reused or recycled and only about 14% going to landfill (Australian Food and Grocery Council, 2005).

A useful sequential approach to reducing waste is illustrated by the 'waste minimisation triangle' (Fig. 10.1). When using this approach, dairy processors need to first look at areas where they can totally eliminate all unnecessary waste. They can then consider further ways to reduce their waste by reusing or recovering product. Once these options have been explored, processors should then consider recycling. The final step, disposal, should only be explored when all other options have been exhausted. Some opportunities to minimise solid waste include supply chain management, value adding, recycling and reuse.

Efficient supply chain management reduces waste by ensuring raw material and product are delivered at the correct time and in the correct quantity and quality. It ensures raw materials and product are appropriately packaged and not spoilt in transit through poor storage or handling, coupled with an efficient inventory tracking system, good storage and handling practices and best-practice procedures in place for temperature monitoring and control for chilled products.

There may also be opportunities to recover valuable by-products that can be either reused on site or sold. For example in cheese manufacture, whey that was previously considered a waste is now recovered using membrane separation to produce lactose (permeate), which can be used in milk powder standardisation,

IP Address: 129.132.208.100




**Fig. 10.1** The waste minimisation triangle.

© Woodhead Publishing Limited, 2010

Downloaded From Vet-x-Books.com

baking, infant formula and pharmaceuticals, and concentrated protein (retentate), which can be used as a food ingredient.

**Case Study 15**
Murray Goulburn in Rochester, Australia, uses membrane technology to process around 800 kL of whey a day to produce whey and lactose powders. Separated water is also recycled, reducing the need for fresh water by up to 70 ML a year (Dairy Australia, 2005).

Recycling involves reprocessing solid waste into another product. An effective recycling system requires good planning and monitoring. For example, general waste and recycling bins should be clearly labelled, preferably colour coded and located close to the site where the waste is being generated. All staff should be trained and records kept on how well the system is working. Results can be used to keep staff informed and motivated on their recycling efforts.

**Case Study 16**
Dairy Farmers in Lidcombe, Australia, conducted a waste assessment and identified that 58% of waste was being sent to landfill that could be diverted through a reuse and recycling system. A recycling system was established which halved the quantity of waste going to landfill and reduced transport and landfill costs by AUS$40,000 a year (Dairy Australia, 2005).

IP Address: 129.132.208.100

Of course, dairy processors should also consider the impact of packaging on the environment throughout the supply chain. Initiatives to reduce solid waste include lightweighting and optimising packaging design to reduce material use. All unnecessary packaging should be removed and products delivered in bulk if possible. Packaging should also not be damaged unnecessarily through poor handling and storage or poorly operated packing lines. In some cases increasing the recycled content can also reduce the impact of packaging on the environment. Reuse options may also exist for organic dairy processing waste including animal feed, composting and soil injection or direct land spreading.

Possible sources of protein and fat for animal feed include separator de-sludge, whey and product and possibly biosolids from wastewater treatment. A risk assessment should be undertaken to determine if there are any possible animal health risks from chemicals and polymers used in wastewater treatment if biosolids are to be used as animal feed. Compactors are also useful for separating organic from non-organic waste and can help reduce transport costs related to disposal. Composting is another option for large amounts of sludge, again if transport costs are not too high or the plant is in a regional area. Plants

© Woodhead Publishing Limited, 2010

Downloaded From Vet-eBooks.com

wishing to compost on site should consider if odour issues are likely to be a problem for neighbouring sites.

It may be possible to apply or inject organic waste directly into or on soils. During the drought the injection of wastewater treatment sludge from Dairy Farmers in Lidcombe, Australia, on farms west of Sydney was considered invaluable. Application rates, of course, are limited by the nutrient requirements of the soil, so the components of the waste must be monitored regularly (Prasad *et al.*, 2004).

## 10.4 Conclusions

The dairy industry is a global industry which is responsible for providing high quality nutrition for much of the world's population on a daily basis. It is an important and far-reaching sector and as such has significant environmental impacts along the supply chain. Application of an 'eco-efficiency' mindset will help to minimise these impacts while at the same time helping to make the sector more sustainable and profitable.

Eco-efficiency starts at the farm. By implementing best-practice farming principles and managing herds for optimum output, farmers can make a significant contribution to all aspects of environmental management while also saving money. Efficient farming practices will ultimately translate to a more reliable and safe milk source for collection. While the authors have not considered transport and HACCP issues in this chapter, tying together a good collection and transport system (which includes good communication with the farmer) and a tightly controlled cold chain will also minimise costs and reduce waste while also ensuring a safer product.

Dairy processors also have many opportunities to minimise environmental impacts by improving the efficiency of their operations while optimising returns. As energy and water costs continue to rise and waste regulations become more stringent, an eco-efficient approach will present environmental and economic benefits for the sector. There continue to be significant opportunities for the sector to coordinate efforts along the supply chain and minimise the total lifecycle impacts through a structured, businesslike approach of implementing eco-efficiency.

IP Address: 129.132.208.100

## 10.5 References

ATLAS COPCO (2003) 'Energy recovery systems' brochure (accessed April 2003).

AUSTRALIAN FOOD AND GROCERY COUNCIL (2005) 'Environmental report 2005', http://www.afgc.org.au/cmsDocuments/EnvironmentReport2005_v2.pdf (accessed 22/05/2008).

BUTLER, R. (2008) 'Transportation Accounts for 15% of Global Emissions', quoting work from Fuglestvedt *et al.* (2007) 'Climate forcing from the transport sectors',

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

*Proceedings of the National Academy of Sciences*, 7–11 January 2008, http://news.mongabay.com/2008/0107-transport.html

CADDET (1996) 'Retrofit cogeneration system at milk processing plant', www.portalenergy.com/caddet/eetb_eut/R257.pdf (accessed 02/2004).

CADDET (1999) 'Thickening and desalinating whey in the dairy industry', www.caddet-ee.org.

CENTER FOR INTEGRATED AGRICULTURAL SYSTEMS (1996) 'Windrow composting systems can be feasible, cost effective', http://www.cias.wisc.edu/archives/1996/06/01/windrow_composting_systems_can_be_feasable_cost_effective/index.php (accessed 18/02/2008).

CENTRAL MURRAY REGIONAL WASTE MANAGEMENT GROUP (2008) 'Remember to recycle right', Victoria, Australia, http://www.cmrwmg.com.au/major_projects.htm (accessed 26/02/2008).

COMPRESSED AIR ASSOCIATION OF AUSTRALASIA, 'Efficient compressed air systems – 3. Compressed air treatment', http://www.amei.com.au/downloads/guide_3.pdf (accessed 06/07/2008).

DAIRY AUSTRALIA (2003) *DairySAT: An Environmental Self Assessment Tool (SAT) for Australian Dairy Farmers*, Melbourne, Australia.

DAIRY AUSTRALIA (2005) 'Australian dairy manufacturing industry state of the environment report', http://www.dairyaustralia.com.au/content/blogcategory/3/245/ (accessed 22/05/2008).

DAIRY AUSTRALIA (2008), 'Saving water in dairies', http://www.dairyaustralia.com.au/content/view/428/339 (accessed 10/02/2008).

DAIRY INSIGHT, ENVIRONMENT CANTERBURY AND DEXCEL (2007) 'A guide to managing farm dairy effluent', New Zealand, http://www.dairynz.co.nz/file/fileid/5374 (accessed 15/02/2008).

DAIRY NEW ZEALAND (2005) 'Snapshot 6: Financial benefits from budgeting for sustainable', in *The Grass Roots of Nutrient Management – Case Studies*, New Zealand, http://www.dairynz.co.nz/page/pageid/2145838021 (accessed 15/02/2008).

DAIRY SA (2008a) 'Reduce, Reuse, Recycle – Dairy Energy Efficiency InforSheet B3: Heating water', http://www.dairyindustrysa.com.au/__data/assets/pdf_file/0008/60938/RRR_InfoSheet_-_Heating_Water.pdf (accessed 25/02/2008).

DAIRY SA (2008b) 'Reduce, Reuse, Recycle – Dairy Energy Efficiency InforSheet B4: Cooling milk', http://www.dairyindustrysa.com.au/__data/assets/pdf_file/0005/60935/RRR_InfoSheet_-_Cooling_Milk.pdf (accessed 25/02/2008).

DAVIDSON, S. (2000) 'Bad breath', *Ecos*, 103 (April–June), 10–11, http://www.publish.csiro.au/?act=view_file&file_id=EC103p10.pdf (accessed 14/02/2008).

DPEC (DAIRY PROCESSING ENGINEERING CENTRE) (2003) *Dairy Processing Engineering Centre Newsletter*, Issue 26, September 2003, www.dpec.com.au.

ECKARD, R. and HEGARTY R. (2004) 'Best management practices for reducing greenhouse gas emissions from dairy farms', New South Wales Agriculture and The University of Melbourne, http://www.greenhouse.unimelb.edu.au/Greenhouse_from_Dairy_Farms.htm (accessed 15/02/2008).

EEBPP (ENERGY EFFICIENCY BEST PRACTICE PROGRAMME) (2000) 'Energy efficient refrigeration technology — the fundamentals', Good Practice Guide 280, www.envirowise.gov.uk.

EECA (ENERGY EFFICIENCY AND CONSERVATION AUTHORITY) (2007) 'Dairy farm cuts hot water bill by 30%', Wellington, New Zealand, http://www.eecabusiness.govt.nz/

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

eib/case-studies/documents/awarua-07.pdf (accessed 26/02/2008).

ELDRIDGE, S. (2004) 'Soil management for dairy and beef cattle grazing', NSW Agriculture, Richmond, Australia, http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0006/167028/soil-dairy-beef.pdf (accessed 05/02/2008).

ENERGY INFORMATION ADMINISTRATION (2003) 'Units for measuring greenhouse gases', http://www.eia.doe.gov/oiaf/1605/archive/gg03rpt/summary/special_topics.html (accessed 10/07/2008).

ENVIROWISE (1999) 'Low-cost process control in the food and drink industry', http://www.envirowise.gov.uk/Ref117 (accessed 06/07/2008).

ENVIROWISE (2003) 'Water loss from leaking equipment', www.envirowise.org/page.aspx?o=doe2836 (accessed 06/01/2005).

ENVIROWISE (2007) 'Benchmarking water use in dairies', http://www.envirowise.gov.uk/EN740 (assessed 22/07/2008).

ETSU (ENERGY TECHNOLOGY SUPPORT UNIT) (1996) 'Spray drying', Good Practice Guide 185, UK Energy Efficiency Best Practice Programme, Harwell, Oxfordshire.

ETSU (ENERGY TECHNOLOGY SUPPORT UNIT) (1998) 'Reducing the energy costs in dairies – A guide to improved profitability', Good Practice Guide 209, UK Energy Efficiency Best Practice Programme, Harwell, Oxfordshire.

HOORMAN, J.J. and CUTCHEON, J. (2005) 'Understanding the benefits of healthy riparian areas', Ohio State University Extension Factsheet, http://ohioline.osu.edu/ls-fact/0001.html (accessed 06/07/2008).

HOPKINS, D. (2004) 'Dairy effluent: Application to pastures', Department of Primary Industries, Australia, http://www.dpi.vic.gov.au/DPI/nreninf.nsf/childdocs/-22C871BE2A0105794A2568B30004D413-17B9D9AE6DF2F16CCA256BC70082448C-F22E47B6332F6F2E4A256DEA0027C63F-F1B089F3EDA5E41ECA256BCF000BBFB5?open (accessed 09/02/2008).

INDUSTRY, SCIENCE AND RESOURCES (2003) 'Energy Efficiency Best Practice Program, Dairy Processing Sector Case Study', http://www.industry.gov.au/assets/documents/itrinternet/DairyProcessingCaseStudy20040206160011.pdf.

JANSEN, A. and ROBERTSON, A. (2001) 'Relationships between livestock management and the ecological condition of riparian habitats along an Australian floodplain river', *Journal of Applied Ecology*, 38, 63–75, http://www.blackwell-synergy.com/doi/pdf/10.1046/j.1365-2664.2001.00557.x (accessed 18/02/2008).

JOHNSON, R. (2007) 'Bedding recovery unit good fit for Adams dairy', *Agri View*, 23 August, Madison, WI, USA, http://www.agriview.com/articles/2007/08/23/features/feature01.txt (accessed 18/02/2008).

KAMRA, D., AGARWAL, N. and CHAUDHARY, L. (2006) 'Inhibition of ruminal methanogenesis by tropical plants containing secondary compounds', *International Congress Series*, 1293 (July), 156–163.

LUNDIE, S., FEITZ, A., JONES, M., DENNIEN, G. and MORIAN, M. (2003) 'Evaluation of the environmental performance of the Australian dairy processing industry using life cycle assessment', Dairy Research Development Corporation Final Report, CWWT Technical Report 02/02, 187 pp, August 2002, Centre for Water and Waste Technology, School of Civil and Environmental Engineering, UNSW, Sydney.

MCDONALD, S. (2006) 'Information notes: Minimizing the effluent stream', Victorian Department of Primary Industries, http://www.dpi.vic.gov.au/DPI/nreninf.nsf/childdocs/-89E7A8DAFEA417624A2568B30004C26A-BBB90E3F477D0B74CA256BC700811C92-7E8749257C5C13324A256DEA0027C5BB-D451336AFA

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

0290F5CA256C1D0002AC9C?open (accessed 18/02/2008).

MCV (MANAGING CLIMATE VIABILITY) (2007) 'Building the kitbag of grazing tools', *Climag*, No. 13, June 2007, Canberra, Australia, http://www.econnect.com.au/pdf/MCV_CLIMAG_Ed13_Lowres.pdf (accessed 09/02/2008).

MDC (MILK DEVELOPMENT COUNCIL) (2005) *Energy Efficiency on Farms*, Cirencester, UK, http://www.mdcfmp.org.uk/uploadeddocuments/Research/MDC_Energy_efficiency_on_farm.pdf (accessed 26/02/2008).

MDC (MILK DEVELOPMENT COUNCIL) (2007) *Effective Use of Water on Dairy Farms*, 40 pp, http://www.dairyco.org.uk/media/10351/effectiveuseofwaterondairyfarms.pdf.

NATIONAL DAIRY ENVIRONMENTAL STEWARSHIP COUNCIL (2005) 'Cost-effective and environmentally beneficial dairy manure management practices', San Francisco, http://www.suscon.org/dairies/pdfs/COST%20EFFECTIVE%20AND%20ENVIRONMENTALLY.pdf (accessed 06/07/2008).

NATIONAL RESEARCH COUNCIL (2001) *Nutrient Requirements of Dairy Cattle*, 7th revised edition, National Academies Press, Washington, DC.

NATIONAL SUSTAINABLE AGRICULTURE INFORMATION SERVICE (2004) 'Rotational grazing – Livestock systems guide', http://attra.ncat.org/attra-pub/PDF/rotgraze.pdf (accessed 05/02/2008).

NEW ZEALAND MINISTRY OF AGRICULTURE AND FORESTRY (2003) 'Abatement of agricultural non-carbon dioxide greenhouse gas emissions', Ministerial Group on Climate Change, Minister of Agriculture and the Primary Industries Council, http://www.maf.govt.nz/mafnet/rural-nz/sustainable-resource-use/climate/abatement-of-agricultural-greenhouse-gas-emissions/httoc.htm (accessed 06/07/2008).

NSW DEPARTMENT OF ENVIRONMENT AND CONSERVATION (2004) 'Dairy gains for the environment through increased productivity', Cleaner Industries Unit, Sydney, Australia, http://www.livingthing.net.au/rc/casestudies/cleanprod/drury.pdf (accessed 05/02/2008).

O'MARA, F. (2004) 'Greenhouse gas production from dairying: Reducing methane production', University College Dublin, Ireland, http://www.wcds.afns.ualberta.ca/Proceedings/2004/Manuscripts/295OMara.pdf (accessed 15/02/2008).

PARMINTER, T. and NELSON, T. (2003) 'Dairy farmers' evaluation of biodiversity practices in South West Victoria', 2003 National Forum, http://www.regional.org.au/au/apen/2003/refereed/094parmintertg2.htm (accessed 06/07/2008).

PGGRC (PASTORAL GREENHOUSE GAS RESEARCH CONSORTIUM) (2006) Press release: 'Big potential in nitrification inhibitors', http://www.scoop.co.nz/stories/SC0611/S00052.htm (accessed 15/02/2008).

PIMENTEL, D. (2006) 'Soil erosion: a food and environmental threat', *Environment, Development and Sustainability*, 8, 119–137, http://www.springerlink.com/content/e344474w45u3811x/fulltext.pdf (accessed 5/2/2008).

PRASAD, P., PAGAN, R., KAUTER, M. and PRICE, N. (2004) 'Eco-efficiency for the dairy processing industry', Queensland, Australia, http://www.gpa.uq.edu.au/CleanProd/dairy_project/Eco-efficiency_manual%202.pdf.

QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES AND FISHERIES (2008) 'Dairybal – a whole farm nutrient and water mass balance spreadsheet', Queensland, Australia, http://www2.dpi.qld.gov.au/environment/1334.html (accessed 15/02/2008).

RTC (RENEWING THE COUNTRYSIDE) (2005) 'Scherping family dairy farm, Minnesota', http://renewingthecountryside.org/component/option,com_smartpages/task,view/category,158/id,164/Itemid,43 (accessed 09/02/08).

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

SEAV (SUSTAINABLE ENERGY AUTHORITY VICTORIA) (2002) 'Energy and greenhouse management toolkit', http://www.sustainability.vic.gov.au/www/html/1938-energy-and-greenhouse-management-toolkit.asp (assessed 06/07/2008).

SEAV (SUSTAINABLE ENERGY AUTHORITY VICTORIA) (2004) 'Infosheet: Combustion trim for boilers', http://www.plantsupport.com/download/CTBB.pdf (accessed 06/07/2008).

SEDA (SUSTAINABLE ENERGY DEVELOPMENT AUTHORITY) (2003) 'Energy smart compressed air calculator', http://www.energysmart.com.au/wes/Displaypage.asp?flash=-1&t=20086161&PageID=53 (accessed 06/07/2008).

SPIELMANN, S. (n.d.) 'Cleaning tubes in boilers, chillers and heat exchangers', Goodway Technologies, Stamford, CT, USA, http://www.goodway.com/company_info/news_events/scale_affects_boiler_performance.aspx (accessed 06/07/2008).

UNEP WORKING GROUP FOR CLEANER PRODUCTION IN THE FOOD INDUSTRY (1999) 'The potential for generating energy from wet waste streams in NSW', NSW Sustainable Energy Development Authority (SEDA), Brisbane, Australia.

VERGÉ, X.P.C., DYER, J.A., DESJARDINS, R.L. and WORTH, D. (2007) 'Greenhouse gas emissions from the Canadian dairy industry in 2001', *Agricultural Systems*, 94(3), 683–693.

VICTORIAN DEPARTMENT OF PRIMARY INDUSTRIES AND UNIVERSITY OF MELBOURNE (2008) 'Dairy greenhouse accounting framework', http://www.greenhouse.unimelb.edu.au/DairyGreenhouseFrameworkv3.xls (accessed 26/02/2008).

VICTORIAN ENVIRONMENTAL PROTECTION AGENCY (2007) 'Bonlac Foods farm management', http://epanote2.epa.vic.gov.au/EPA/Publications.nsf/7dd91371df0bd0654a256ce9001f4ac1/71cc44f913488a33ca2572f9000afd74/$FILE/1130.pdf (accessed 18/02/2008).

WATSCHKE, T.L. (1998) 'Proper use of fertilizers minimizes environmental effects', http://www.grounds-mag.com/mag/grounds_maintenance_proper_fertilizers_minimizes/ (accessed 06/07/2008).

WOODHOUSE, J. (2007) 'Food miles and food exporting', Westwick-Farrow Pty Ltd, New South Wales, Australia, http://www.foodprocessing.com.au/feature_article/article.asp?item=1535 (accessed 24/02/2008).

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

# 11

# Improving organic milk

**R. Weller, Aberystwyth, UK**

**Abstract:** The chapter discusses the key factors influencing the quality of organic milk and the management and husbandry techniques available on dairy farms for improving quality. The key factors include the requirement to feed high forage diets, the importance of dietary energy, grazing strategies for clover-based pastures and minimising health problems. Cropping options for improving the nutritive value of organic rations and the influence of the season of calving on milk quality are also reviewed.

**Key words:** organic dairy systems, organic milk, feed energy, high forage diets, improving the quality of organic milk.

IP Address: 129.132.208.100

## 11.1 Introduction

The major differences in milk quality between organic and conventional systems are attributable to the variation in the type and quality of feeds that are included in the diet of the dairy cow, including the production of organic milk from high forage diets. One of the biggest challenges for the organic dairy farmer is to implement and manage a system that will ensure a consistent production of quality milk throughout the year, from diets that often include a less varied range of constituents compared with those used in the formulation of diets for conventional herds. As forages differ widely in nutrient balance and consistency when compared with concentrate feeds, the quality of organic milk is potentially more variable than that of milk produced from cows in conventional systems fed diets with higher concentrate content. The key factors affecting milk quality during the year are the changes in the quality of feed available for formulating diets, the change from the grazing of fresh herbage to the feeding of conserved

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

forages, the stage of lactation and the influence of the calving pattern on the season of peak milk production.

The production of organic milk is influenced by a number of key factors, including both the standards defined for organic milk production and the need of both the liquid and processing markets to meet consumer demands and expectations. In addition the influence of the type of system, climatic conditions and complexity of the cropping strategy to produce high quality feed for the dairy herd also affects both the level of production and the quality of organic milk. Organic dairy systems vary in size and their approach to management and husbandry options, with a range of systems managed on different farms both within and between different countries. Surveys of organic dairy farms show that, in relation to milk quality, there are large differences between individual farms that are attributable not only to genetic differences in the herd profile but also to management and husbandry factors. While many farms manage to consistently produce high quality milk throughout the year, others record seasonal variations, including the production of milk that is below the standards required for both the liquid and processing markets. For the majority of organic farms the aim of producing quality milk needs to be achieved within a system that also maximises profitability to provide a satisfactory financial return on the capital that has been invested in the system.

Organic dairy systems include the more extensive and sustainable systems aiming for a high level of nutrient self-sufficiency and efficient nutrient utilisation within a management strategy that requires a more complex crop rotation to produce both forage and concentrate feeds. Other systems aim to maximise the output of milk and financial viability per hectare by only growing forage crops in a basic rotation, maintaining a higher stocking density, and purchasing significant quantities of concentrate feeds to ensure diets fully meet the nutritional requirements of the dairy cow. For many farmers the most practical option is a compromise between the above contrasting systems, avoiding both the limitations of a fully self-sufficient system and the total reliance on the purchase and importation of concentrate feeds that vary in both availability and price.

The chapter will address many of the factors that influence the quality of organic milk and identify options for improving quality within the standards defined for organic milk production, while ensuring the system achieves financial viability.

## 11.2 The key factors affecting the quality of organic milk

### 11.2.1 The limitations of the organic standards

The standards defined for organic milk production require a minimum inclusion rate of 60% forage in the total diet. Depending on the stage of lactation and also the availability and cost of different feeds used for formulating the diets, forage can contribute up to 100% of the total feed. Compared with rations formulated

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

for conventional herds, there is a more limited range of ingredients that can be included in organic rations and a greater risk of nutrient imbalance. Both these factors have led to a number of studies showing nutritional deficiencies and an increased risk of lower milk protein concentrations to be a problem on some farms, particularly during the early lactation period (Weber *et al*., 1993; Weller and Bowling, 2004; Nauta *et al*., 2006). The risk of nutritionally related problems is more critical when the level of milk production increases; forage quality decreases and both the energy density and energy–protein balance of the diet become unbalanced. Organic milk also needs to be produced from systems that utilise environmentally sustainable production methods, efficiently recycle nutrients within the system and have a low use of non-renewable resources (Zollitsch *et al*., 2004).

As shown by the incidence of contagious sub-clinical mastitis in some herds, managing herd health status without the routine use of long-acting antibiotics has been found to be more challenging on some organic dairy farms and has led to increased health problems and lower quality milk, particularly when the use of alternative treatments has not been widely adopted.

### 11.2.2 The implications of meeting the requirements of the commercial market

The challenge for organic dairy systems is not only to produce milk within defined standards from a system that is financially viable and minimises environmental impact, but also to meet the demands of the commercial market. Consumers require a supply of fresh milk on a regular basis, therefore a key objective in the management of the herd is to sustain a consistent output of high quality milk throughout the year. As the availability and quality of the different grazed and conserved forages change significantly during the year, the challenge of providing balanced diets for the dairy cow requires an evaluation of the cropping strategy and other management options to meet the requirements of the commercial market.

IP Address: 129.132.208.100

### 11.2.3 Limitations on the individual organic dairy farm

Studies have shown wide variations in the proportion of organic milk that is produced from the forage component of the diet, with large differences recorded between organic farms in how efficiently they utilise forage and their level of dependency on concentrate feeds to achieve optimal yields of high quality milk. These results suggest that on many individual farms there is scope to improve the management of the herd in relation to the type and quality of forages that are produced, the strategies used for grazing and forage conservation, and the efficiency with which the forages are utilised in relation to the formulation and feeding of high forage diets. Large differences have also been recorded between farms in their approach to husbandry practices and the management of the herd, indicating that not only diet but other key factors, including genetics and

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

environmental issues, may also be limiting the potential of the herd to maximise the production of quality milk.

The effect of the standards defined for organic milk production may be more critical for farms in less favourable climatic areas that are unable to grow a wide range of crops, as their ability to produce quality milk from nutritionally balanced diets is more limited unless high quality herbage can be produced in adequate quantities, both during the grazing season and as conserved forages for feeding during the winter period of housing. The increased complexity of establishing and maintaining a more complex cropping strategy to ensure high quality feed is available throughout the year may not be either practical or financially viable on smaller farms with limited labour and machinery resources, unless the cost of employing contractors can be justified.

## 11.3 Management and husbandry techniques to improve the quality of organic milk

### 11.3.1 The nutritional balance of the diets

Maintaining the production of quality milk is dependent on maximising the intake of high quality diets and achieving compatibility between the genetic merit of the cow and nutritionally balanced diets that are appropriate for the different stages of the lactation cycle. The plane of nutrition is a critical factor influencing both the composition and the processing properties of milk, with adequate feed energy essential in the production of milk with an acceptable protein concentration and a milk fat to protein ratio < 1.5. In a review of 13 studies, Emery (1978) found increasing feed energy intake and milk protein concentration to be positively correlated and attributable to the increasing proportion of concentrate feeds in the diet. Sporndly (1989) reported an increase of 10 MJ of metabolisable energy (ME) per day, increasing milk protein content by 0.03–0.05% points. As forage contributes between 60 and 100% of the total feed in the diet of the organic dairy cow, one of the major challenges is to formulate high forage diets with an adequate energy concentration. As the energy value of the majority of forages is generally lower (<11 MJ of ME per kg DM) than that of concentrate feeds (>13 MJ of ME per kg DM), the strategy on organic dairy farms has to focus on ensuring that sufficient quantities of high quality forages are not only grown but also consumed in sufficient quantities by the dairy cow throughout the year. Although grazed herbage generally has a higher energy value compared with conserved forages, a shortage of energy has been found to be a limiting factor during the grazing season as well as when diets based on conserved forages are fed. While many organic herds are able to achieve milk yields of 4000–5000 kg per cow from the forage component of the diet, others fail to reach these levels and rely on concentrate supplementation to achieve satisfactory lactation yields, indicating a need to improve the production and efficient utilisation of forages.

Achieving a satisfactory output of quality milk during the early lactation period is a major challenge for many organic dairy farmers feeding high forage

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

diets, with maximising energy intake the key challenge. Early lactation is inevitably a period of negative energy balance for the majority of dairy cows, and the risk of a severe deficit of nutrients will be at a maximum during the first eight weeks of lactation (Zollitsch *et al.*, 2004). This is primarily due to peak feed intake not being normally achieved until three to four weeks after the attainment of peak milk yield (Thomas *et al*., 1999). The magnitude and length of time of the negative energy balance will be influenced to a greater extent by variability in feed intakes and increasing genetic merit, with high genetic merit cows producing an extra 6.6 kg of milk per day but increasing feed intake by only 0.8 kg per day and maintaining a negative energy balance for the first 20 weeks of lactation (Villa-Godoy *et al.*, 1988; Gordon *et al*., 1995; Beever *et al.*, 1998).

One of the problems when formulating high forage diets is the significant changes that occur when the diets change from grazed herbage to conserved forages, temporal changes in the components and quality of grazed swards, and the significant differences between the quality and physical attributes of different conserved forages. Although supplying sufficient feed energy is the main limitation in the formulation of organic diets based on conserved forages, a shortage of protein has also been found to limit the level and quality of milk produced (Thuen *et al*., 2002), and the potential contribution of different annual and perennial legumes (e.g. white clover, red clover, Lucerne, crimson clover, vetch) as valuable sources of feed protein needs to be recognised. On all farms there will be differences in the quality of the different forages that are available annually. Knaus *et al*. (2001) concluded that to achieve a lactation yield of 7000 kg per cow the proportion of concentrates in the diet would need to increase from 26 to 40% when low rather than high quality forage was fed, although the increased concentrate supplementation would not reduce the risk of a high body weight loss (−52 kg) through the mobilisation of body fat during the early lactation period. The benefits of improving forage quality were also reported by Kreuzer *et al*. (1996), with a 5.5% increase in metabolisable energy from 5.25 to 5.54 MJ net energy for lactation improving both the composition and processing properties of milk. Therefore, it is essential that the highest quality forages on the farm are fed to cows during the early and mid-lactation periods, with the more mature and lower quality forages fed during the less nutrient-demanding periods of late lactation and the dry period. While mixed grass and white clover swards continue to be the main forage crops for grazing and conservation, alternative forages, including high energy fodder beet and forage maize and high protein crops such as lucerne and red clover, will improve feed intake (Phipps *et al*., 1995) and optimise the energy supply of the high forage diets for producing quality milk.

IP Address: 129.132.208.100

### 11.3.2 Organic forage cropping options

The production of milk in organic dairy systems is primarily from forage grown in mixed swards based on grass and white clover as the main species in reseeded

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

leys and permanent pastures. However, other high protein and high energy forage crops, grown either as a monoculture or in a mixture, have the potential to improve both the nutritional value of the diets and the quality of milk produced. These crops are fed primarily as conserved forages in silage-based diets fed during the winter period of housing but also in periods of reduced herbage availability during the grazing season. While some crops have now become more widely established, the potential benefits from other crops have not been fully exploited. As nitrogen is potentially limited within the closed nitrogen cycle on organic farms, when new crops are established a balance needs to be maintained between the different N-producing legumes and N-demanding species that are included in a crop rotation.

*Higher protein forages*

Different legumes are important not only for their contribution of N via fixation but also for their high intake characteristics and supply of feed protein. Compared with grasses, legumes have a higher digestibility value due to a higher cell content to cell wall ratio. While white clover is the primary legume grown in grazing swards on organic dairy farms, the potential benefits of other annual and perennial legumes as crops for conservation, including red clover, forage peas, crimson clover, lucerne and vetch, have not been fully exploited. Although a shortage of feed energy is often a problem with organic diets based on conserved forages, a shortage of protein also occurs when silage is made in the spring.

Red clover crops are grown for two to three years, primarily as a conservation crop and either as a monoculture or in a mixture with grass species, including Italian ryegrass, hybrid ryegrass, perennial ryegrass or timothy. Red clover produces high yields of a palatable feed that can improve feed intake and both the milk protein concentrations and polyunsaturated fatty acid content of milk (Al-Mabruk *et al*., 2004). The energy value of red clover silage ranges from 9.8 to 11.4 MJ/kg of dry matter (DM), and the protein content ranges from 14 to 19%. To avoid the risk of increased oxidative deterioration of milk and reduced shelf-life when red clover silage is the sole forage included in the diet (Al-Mabruk *et al*., 2004), the inclusion rate should be limited to 50% of the total forage component.

Lucerne is a deep-rooting perennial crop with the potential to provide a high protein forage crop in areas of low rainfall. Once established a lucerne ley will produce reliable yields over a four-year period. Crimson clover, forage peas and vetch are annual legumes whose potential to increase the protein content in both short-term leys and mixtures with whole-crop cereals has not been fully exploited. The problem of low protein when the first silage cut is taken in the spring from grass-dominant swards can be overcome by including a combination of three legumes (crimson clover, red clover, vetch) in short-term leys.

Kale is a fast-growing crop and a palatable feed with both high protein (14.7–18.8%) and high energy (11.0–12.7 MJ/kg DM) contents, which is suitable for grazing by dairy cows when the productivity of grass plus white clover leys declines from late summer onwards. However, the efficiency of utilisation of the

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

grazed crop can be as low as 50–60% and high intakes of kale can cause tainting of milk unless limited to 35% of feed intake.

*Higher energy forages*

While many organic farms rely on only grass plus legume silages as the conserved forage for feeding during the winter period, the feeding of other forages with a higher energy to protein ratio has the potential to improve the nutritional balance of the diet, increase total forage intake and avoid a sharp decline in milk quality when the diet of the herd changes from grazed herbage to silage-based diets.

Fodder beet and forage maize are both high energy crops (>11 MJ of ME/kg of DM) that can be grown within an organic rotation and their inclusion provides essential feed energy, enhances forage intake by improving the palatability of the diet and improve milk protein concentrations during the housing period. However, producing good yields of both crops is dependent on effective weed control and suitable weather conditions during the harvesting period, with a DM content of 30–35% at harvest essential to ensure that high quality maize crops with a >50% grain content are ensiled. In formulating organic diets that include either forage, it is important to note that both crops are low in both protein and mineral contents.

In addition to producing grain for feeding and straw for bedding, cereal crops can also provide additional forage energy when the whole plant is harvested and ensiled as whole-crop cereal silage at 35–45% DM and with the grain at the soft dough/soft 'cheddar' stage. Whole-crop cereals are cut three to four weeks prior to normal grain harvest and often only 16 weeks after the crop is sown. Compared with both fodder beet and maize silage, whole-crop cereal silage has a lower energy value (9–10 MJ of ME/kg DM) than either fodder beet or maize, but cereals can be more widely grown, produce consistent yields and provide palatable feed to complement high protein grass plus legume silages. Cereals also provide an effective cover crop when new grass plus clover leys are being established, with minimal weed problems compared with those found in fodder beet and forage maize crops, particularly when crops are under sown. In addition to being grown as a monoculture, cereals can be grown in mixtures with protein crops, including forage peas and vetch, leading to higher energy and protein forage and providing extra nitrogen via fixation for improving total forage production within the system.

*Improving the quality of reseeded leys*

Increasing the species diversity within a sward based on perennial ryegrass and white clover has the potential to improve milk quality due to increased palatability, enhanced herbage intake and improved nutrient balance. While higher fertiliser-N inputs on ryegrass-dominated swards are common on many conventional dairy farms in lowland regions, under the moderate-N inputs in organic systems other grasses including timothy, cocksfoot and meadow fescue are suited to specific environmental conditions and can produce yields comparable

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

with ryegrasses. For example, timothy grass is highly palatable, cocksfoot is suited for drier areas, and meadow fescue is productive on heavier soils in wetter regions. Herb species, either sown in new leys or maintained in permanent pastures, have a valuable role in organic diets due to their high mineral concentration. Chicory and ribgrass plantain are two herbs whose value has been recognised. However, the survival of herbs in a grazing sward is influenced both by the grazing strategy and by competition from more aggressive species in the sward.

*The important role of permanent pastures*

While many studies on organic milk production focus on the production of herbage from reseeded leys and the benefits of a crop rotation, the role of permanent pastures is also essential in many systems and their important contribution to the production of quality milk should not be underestimated. Many of these pastures contain a wide range of plant species that are adapted to the local environmental conditions. These pastures also affect milk quality, including influencing the production of milk that characterises individual cheeses with different sensory properties from lowland and upland regions, attributable to the range in the botanical composition of the individual pastures. In a long-term pasture where the botanical composition is stable, the optimum composition has been reported to be 50–60% grasses, 20–30% legumes and 10–20% other species. Maintaining a balance between different species in the sward will be influenced by both the stocking density and other management practices that have a major influence on the stability of the botanical composition and the potential of the pasture to provide herbage that leads to good milk quality.

IP Address: 129.132.208.100

*Improving silage quality on organic farms*

Silage is the main conserved forage fed to organic dairy herds during the winter housing period. It influences feed intake and the quality and quantity of milk produced. It is primarily made from clover and other legumes, ensiled either alone or more commonly in a mixture with grass species. As legumes have lower sugar contents and a higher buffering capacity than grasses harvested at a similar stage of maturity, wilting low DM crops prior to ensiling and the use of a biological additive will improve the fermentation process. Other crops for ensiling include forage maize and whole-crop cereals, with maize silage readily fermented without an additive due to the low buffering capacity and rapid decline in pH when the crop is ensiled.

While the nutritive value of ensiled crops is lower than that of the fresh material, ensiling at DM contents >23–35% will ensure that nutritive losses due to the production of effluent are minimised, and will also avoid the problem of leaf losses from high DM legume crops due to mechanical damage pre-ensiling. Silage made from leafy mixed grass and legume crops will lead to higher intakes and improved milk protein compared with ensiling more mature crops with high stem contents, due to a greater reduction in particle size from mastication and a more rapid rate of passage out of the rumen of the more digestible forage.

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

However, leafy crops have a lower DM content, require more wilting prior to ensiling and produce a lower yield per hectare than more mature crops.

### 11.3.3 Grazing strategies and the role of white clover

During the grazing season organic milk is produced from diets that are based partially or wholly on grazed herbage from mixed grass and white clover swards produced from both reseeded leys and permanent pastures. These swards have the potential to lead to higher intakes of herbage during the grazing season, due to the beneficial contribution of the clover plants and a greater persistency of quality that is attributable to the increasing proportion of clover as the grazing season progresses. Maximising milk production from grazed herbage has been found to improve milk quality when compared with diets based on conserved forages, including increasing vitamins A and E concentrations, carotenoid content and up to 500% more of the anti-carcinogen conjugated linoleic acid (Dhiman *et al*., 1999). Milk quality is influenced by the quantity of available herbage and also the composition and maturity of the sward. Cows grazing leafy swards will produce milk with a higher content of conjugated linoleic acid compared with those grazing more mature swards with a high stem to leaf ratio (Bell *et al*., 2006). Organic herds calving in the spring are well placed to benefit from grazing mixed grass and white clover pastures. However, it is widely acknowledged that achieving consistent levels of high quality milk is limited by the constraints of accurately determining the nutritive quality and intake of grazed herbage, and is more difficult when compared with diets based on conserved forages where the consistency of the quality and intake of the silage-based diets are often less variable. Therefore, organic farmers calving cows in the spring and producing the major part of the annual milk output from grazed herbage need to be aware of two key factors affecting production.

The first factor is the potential lack of milk persistency and shortened lactations that lead to a reduced output of milk quality components due to farmers being unable to accurately estimate both the nutritive value and actual intake from the herbage, leading to supplementary feeds being required to balance the diet and allow the cow to achieve her genetic potential. The intake of grazed grass herbage has been found to support milk yields of *ca.* 25 kg/day from cows consuming 15.0 kg DM/day (Mayne and Peyraud, 1996). However, to achieve milk yields of >30 kg/day from higher genetic merit cows, Kolver and Muller (1998) found that even with herbage intakes of 19 kg DM/day the inclusion of a supplementary feed was required, and Mayne and Peyraud (1996) concluded that to maximise herbage intakes the grazing strategy should include offering >18 kg DM of organic matter/day, or *ca.* 21–22 kg DM/day from a dense sward with a surface height of 12–14 cm. Ensuring swards are not grazed to a sward height below 7–8 cm also enables the cow to achieve satisfactory intakes by allowing the selection of the more nutritious plant parts. Organic swards with satisfactory clover content have the potential to increase herbage intakes above the levels achieved with pure grass swards but are still unlikely to

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-x-Books.com

meet the requirements of the higher yielding cow. It is important to note that at the same sward height, the quantity of herbage per unit area on organic farms will be about 20% lower during the grazing season compared to those recorded on conventional dairy farms. Therefore, the stocking density on organic farms also needs to be lower to ensure optimal yields of quality milk are produced.

*Protein changes in the composition of organic swards*

The second factor is the change in the energy and protein concentrations of the diet as the ratio of grass to clover changes during the grazing season. The energy to protein balance of the diet and its influence on milk composition will be affected by simultaneous changes in both the botanical composition of the sward and protein content. While the optimum clover content in the total DM is approximately 30%, during the grazing season the proportion of white clover in a mixed grass and white clover sward increases, reaching a peak in midsummer that may result in up to 70% clover in the total DM content of an individual sward. In addition, there is a temporal change in the protein content of the sward throughout the grazing season, with the protein content of the white clover component increasing from 22 to 31% and the grass component from 11 to 24% (Weller and Cooper, 2001). By early July the protein in the herbage, including a high proportion of rumen-degradable protein, is significantly above the cow's nutrient requirements and will reach 25% by the autumn, potentially leading to poor protein utilisation by the cow as extra feed energy is required for urea excretion rather than being converted to milk protein. When herbage supply is plentiful, a preference for clover rather than grass plants will increase the intake of protein even further. For the high yielding cow receiving a buffer feed (e.g. silage or concentrate supplement) that has a high energy/low protein value, the diet can be balanced, but for cows not being offered a supplementary feed the protein content of the diet will be too high during periods of the grazing season. A failure to maintain the correct energy to protein balance in the diet, resulting in the intake of excess protein, affects milk quality both directly and indirectly due to reduced conception rates and a change in the annual calving profile of the herd.

Although grazed forages with a high protein content (organic high clover swards, conventional spring grass receiving a high fertiliser-N input) have been found to increase the protein content of milk, they also led to an increase in blood urea levels and a higher protein content in the diet can reduce reproductive efficiency and also increase lameness compared with lower protein diets (Butler *et al.*, 1996; Manson and Leaver, 1988; Plym Forshell, 1994; Chamberlain and Wilkinson, 1996). Therefore, the potential risk, irrespective of the calving pattern of the herd, when non-pregnant cows graze protein-rich swards, including grass/clover mixtures, and do not receive any supplement of a high energy/low feed or only a small quantity (e.g. spring calving organic herds), should be assessed. Measuring the urea content in the milk is a useful reactive indicator of when the diet reaches excess protein that leads to energy to protein imbalance.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

*The influence of stocking density and type of grazing system*

Stocking density is also an important factor during the grazing season and has been found to affect the quality and processing characteristics of milk when grazed herbage is the sole feed. In managing grazing swards to maximise the level and quality of organic milk, changing the stocking density will affect the output per cow, with the effect being more significant for higher yielding cows. While increasing the stocking density leads to maximum milk output per hectare, yield per cow and output of milk solids are reduced and the risk of negative feed intake increased. To maintain milk quality and also avoid adverse changes in the botanical composition of the sward, either the total grazing area needs to be increased or the density of cows needs to be decreased during periods when herbage growth is slower.

Grazing systems used on organic farms include strip grazing the grass plus white clover swards with an electric fence, the rotational grazing of paddocks in a 28–35 day cycle, and the continuous grazing of the same area throughout the grazing season. The type of grazing system is unlikely to have a major influence on milk quality when compared with ensuring that the appropriate stocking density is maintained and that sufficient quantities of leafy, high quality herbage are available. However, a rotational grazing system has been found to lead to a more uniform pattern of herbage intake that will minimise the daily variations in the output and concentration of milk components. The leader/follower strategy within a rotational grazing system, where the highest yielding group of cows graze ahead of the lower yielding group, has the potential to minimise problems of feed energy deficiency by allowing the high yielding cows to selectively graze the more nutritious plant parts, resulting in an increase in both the quality and quantity of milk produced. Unless the appropriate stocking density is maintained during the grazing season, the proportion of clover can adversely decline when swards are continuously grazed, and a silage cut will become necessary in the later part of the summer when rapid clover growth occurs, to ensure the productivity of the sward and output of milk are sustained.

As the clover content of the grass plus white clover swards increases during the season, the fibre content of the total diet decreases, leading to the ingestion of more forage that is rapidly digested. This leads to an increased risk of bloat that occurs on some organic farms despite high standards of grazing management. The risk of bloat will be minimised by ensuring hungry cows never have access to clover-rich pastures and the herd has a similar quantity of fresh herbage each day, with additional sufficient long fibre also made available from other feeds. Both lucerne and red clover can be occasionally grazed to improve forage intake and quality when the productivity of grazing swards is low. For example, in France, lucerne varieties selected for persistency and grown in association with cocksfoot have proved to be successful under grazing management (Charrier *et al*., 1993). However, there is a high risk of bloat to the herd and also declining yields when either crop is grazed frequently.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

*Buffer feeding as a management option during the grazing season*

Seasonal changes in the quality and quantity of herbage from grazed swards are inevitable on organic dairy farms, often leading to significant challenges in relation to maintaining both the yield and quality of milk components. Variations in herbage availability, sward composition (e.g. grass to white clover ratio, DM content) and nutritional value occur throughout the grazing season, and any adverse effects of weather (wet and/or windy conditions, heat stress) on milk production can be minimised by offering a supplementary feed (concentrates, alternative forage) in addition to the herbage that is available. Offering a supplementary feed has the potential to minimise the risk of an imbalance in the protein to energy ratio of the diet and low dietary fibre and low milk protein concentrations.

Buffer feeding has the potential to overcome the problem of oversupply of protein relative to feed energy that occurs when grass plus white clover swards are grazed, with energy rich/low protein feeds such as maize silage, whole-crop cereal silage, fodder beet or concentrates – all suitable energy sources (Zollitsch *et al*., 2004). The quality of the buffer feed is important, and Phillips (1988) reported that milk protein content can be reduced when grazed herbage is supplemented with silage that is of a lower quality than the grazed herbage.

A lack of fibre is a common problem in conventional systems when cows graze leafy grass pastures, leading to reduced saliva production and chewing activity, the risk of ruminal acidosis and a sharp reduction in the fat content of milk. White clover plants have markedly lower fibre contents than grass, and in organic systems fibre deficiency is likely to occur in the grazing season. The buffer feeding of palatable forage (good quality hay or silage) will provide extra fibre to minimise the risk of changes in milk quality.

IP Address: 129.132.208.100

### 11.3.4 Optimising feed quality with the cow's peak nutrient requirements

Whether milk is directly processed on the farm or sold to a milk cooperative, the aim should be to maintain a consistent quality throughout the year. The season of calving and the start of lactation within the herd have a direct influence on the supply and quality of organic milk during the year and can also have a major effect on the requirement for different forage and concentrate feeds. The types of feeds that are available during the early lactation period and the production costs per unit of milk are important factors to consider when deciding on the most viable calving season for the organic herd; however, the major factors influencing the decision are the destination of the milk from the herd and the effect of the calving season on the financial viability of the system.

To achieve a consistent output of milk throughout the year, a year-round calving season is the main choice for most organic dairy farmers, with the milk either sold directly on a regular basis from the farm or purchased by a cooperative for the liquid market. Therefore, the main priority is to produce a consistent quantity of high quality milk throughout the year, irrespective of the availability of individual feeds at specific times of the year. This is a particular

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

challenge in herds calving cows during midsummer when herbage growth is markedly lower. An alternative option to maintain a relatively stable output of milk throughout the year is to divide the herd into two separate groups, with the groups calving in short eight-week periods in both the autumn and spring. This results in the lower daily yields but higher milk solids of the cows in late lactation being balanced by the higher yields and lower milk solids output of the newly calved cows.

For those farms producing milk for the processing market or selling to larger cooperatives, there may be more flexibility in when the milk is produced, and this provides the opportunity to calve all cows within a short time period, for example block-calving within one annual eight-week period. This strategy offers two choices, with the more extensive systems feeding a low proportion of concentrates to spring calving cows and maximising the output of the annual milk production from grazed herbage, while more intensive systems block-calve in the autumn period and feed diets based on a combination of conserved forages to complement grass plus clover silages and a greater proportion of concentrates in the total diet. A spring calving season offers the opportunity of minimising feed costs as the cow's peak nutritional requirement is more easily matched by the availability of the cheapest form of forage, i.e. high quality fresh herbage during the main part of the growing season.

To maximise the utilisation of the high quality herbage for milk production requires the cows to calve prior to changing from a diet of conserved forages fed indoors to grazing high quality herbage. However, spring-calving cows are more vulnerable to the adverse effects of less favourable and unpredictable weather conditions during the spring on milk yield and quality, yield persistency and total lactation yield, due to a more erratic pattern of daily DM intake, reduced milk fat concentrations and higher somatic cell counts. Systems based on the block-calving of the herd in the autumn have the potential to produce higher yields of milk solids from more consistent daily feed intakes and achieve greater milk persistency compared with spring-calving herds, as the diet can be more carefully controlled. However, the system is dependent on feeding a higher proportion of concentrates in the diet, producing high quality conserved forages to ensure adequate intakes are achieved, and an acceptance that the production costs per unit of milk are higher than for milk produced from grazed herbage.

IP Address: 129.132.208.100

### 11.3.5 Minimising the risk of health and welfare problems

*Somatic cell counts*

High standards of hygiene and animal husbandry are critical in all dairy systems. In organic systems where herd health status is achieved without the routine use of conventional medicines, the standards need to be extremely high to minimise the risk of health problems in the herd and ensure high quality milk is produced. The importance of high standards was reported by Ellis *et al*. (2007), who found that cow cleanliness in organic dairy herds had a greater negative influence on milk somatic cell counts compared with conventional herds. Increases in the

© Woodhead Publishing Limited, 2010

Downloaded from Vet-e-Books.com

occurrence of individual health problems, including both clinical and sub-clinical mastitis, have the potential to adversely affect on milk quality. A number of surveys of the health status of organic dairy herds have shown that in practice some organic farms manage to achieve high standards and a low incidence of problems, while others find controlling herd health more challenging. It is essential to employ fully trained staff committed to maintaining high standards of husbandry within the standards defined for organic milk production to minimise the risk of both contagious (e.g. *Staphylococcus aureus*) and environmental pathogens affecting herd health and milk quality, irrespective of the size of the herd or the type of management system.

An important challenge for organic dairy farmers is to ensure that somatic cell counts in the milk are maintained at a satisfactory level, as a high cell count is an indicator of the health status of the udder, with both Renau (1986) and Urech *et al*. (1999) reporting that healthy cows have an average of <200,000 cells/ml. High cell counts reduce the stability of the milk and its shelf-life. Processing milk with high cell counts results in increased costs due to the loss of yield (casein) during the cheese-making process and reduced quality due to increased proteolysis during storage (Auldist *et al*., 1996; Klei *et al*., 1998; Ma *et al*., 2000).

Housing cows in straw-bedded yards rather than cubicle sheds is a standard practice on many organic dairy farms and increases the risk of mastitis and higher levels of somatic cell counts due to environmental pathogens, with *Streptococcus uberis* the main pathogen (Blowey and Edmondson, 1995). Krutzinna *et al*. (1996) reported that the somatic cell counts were significantly higher when organic herds were housed in straw yards rather than cubicles (352,000 vs. 258,000 cells/ml). Weller and Bowling (2000) also reported markedly higher average cell counts for organic herds housed in straw yards (275,000 cells/ml) compared with the lower cell counts (216,000 cells/ml) of herds housed in cubicle sheds. In both studies there were large differences between herds in the average somatic cell content of the milk, with the average cell count from many organic herds above the threshold at which a decline in cheese quality and yield occurs. Key factors to maintain low cell counts when herds are housed in straw-bedded yards include frequent renewal of adequate quantities of straw and the complete cleaning out of the yards at frequent intervals during the period of housing.

IP Address: 129.132.208.100

*Metabolic disorders*

The risk of negative energy balance is critical during early lactation (Duffield *et al*., 1997; Heuer *et al*., 2000) and can lead to both clinical and sub-clinical ketosis. The magnitude of the negative energy balance is potentially greater in organic systems feeding high forage diets. This is attributable to an imbalance between the cow's nutrient requirement and the energy density of the diet, and also the lower energy density of forages compared with concentrate feeds. Providing a balanced diet with sufficient metabolisable energy is essential to prevent excessive mobilisation of body fat (Roepstad *et al*., 1989), suppression

© Woodhead Publishing Limited, 2010

of the immune system and an increased risk of ketosis that not only leads to lower milk quality and yield but also predisposes the cow to the occurrence of secondary health problems, including lameness, mastitis and infertility. In addition, ketosis affects the financial viability of the organic herd due to a reduction in milk income and an increased culling rate due to infertility, particularly when silage diets are fed. The results from a number of studies show that the risk of ketosis problems is reduced by feeding >5 kg of concentrates/day and including only high forage levels in the diet, including forage maize, fodder beet or fresh herbage from leafy swards (Weber *et al*., 1993; Vaarst and Enevoldsen, 1994; Weller and Bowling, 2004).

*Reducing stress and behavioural problems*

In organic systems there is a need for a high standard of management to ensure that the feeding of high forage diets does not increase the potential risk of stress that can lead to both primary and secondary health problems and a decline in milk quality. Milk quality can be adversely affected by stress, including an increase in somatic cell count levels and lower protein concentrations. Factors increasing stress include sudden environmental changes (e.g. the change from housing to grazing), the occurrence of adverse weather, sudden dietary changes (from pre- to post-calving diets) and behavioural problems associated with either inadequate feeding facilities or mismanagement of groups within the herd.

Studies have shown that a significant proportion of the health and stress problems in a dairy herd occurs during the six-week transition period from pre- to post-calving, potentially adversely affecting milk quality for a significant period after calving. The transition period often includes a marked change in the type and quality of ingredients that are fed to the cow post-calving. Both the physical stress of calving and the need of the digestive system to adapt to the changes in the diet need to be considered when the pre- and post-calving organic rations are formulated, as both the type of diet fed during the dry period and the adaptation of the digestive system from the pre-calving to post-calving diets influence the performance of cows in the subsequent lactation (Goff and Horst, 1997; Ryan *et al.*, 2003). During this period the periparturient diseases that occur in conventional dairy systems also occur in organic systems (Bosberry and Dobson, 1989; Offerhaus *et al.*, 1994; Weller and Bowling, 2000), including milk fever, retained foetal membranes and endometritis.

As organic herds increase in size the risk of stress to the individual animal, particularly first-lactation heifers, from social dominance and increased competition between cows becomes more important, including problems of displacement from the feed barrier and reduced forage intake. Avoiding large numbers of cows within each group, restricting the transfer of cows between groups, separating mature cows from first-lactation heifers, reducing the stocking density and increasing the available width per cow at the feed barrier (silage-based diets) or electric fence (strip grazing) provide options for maximising forage intakes within the herd and ensuring quality milk is produced.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

### 11.3.6 Genetics compatible with organic systems

When selecting a suitable type of cow for an organic system, the farmer needs to consider the type of management system and whether the diversity of crops being grown and the degree of reliance on imported feeds will provide diets to achieve the production of quality milk from higher genetic merit cows or are more suited to lower genetic merit cows producing quality milk from lower lactation yields.

Producing quality milk in more extensive organic systems whose strategy is to produce <6000 litres of milk per cow, primarily from grazed herbage supplemented with minimal concentrate supplementation (<0.5 tonnes per cow), including 5000 litres from the forage component of the diet, will generally be more achievable from cows of lower genetic merit. In more intensive organic systems aiming for higher yields per cow (>8000 litres), high quality milk can be produced from diets based on mixed grass plus legume silage that are nutritionally improved by the inclusion of high energy-rich forages (fodder beet, maize silage) and a higher level of concentrate supplementation (>1.5 tonnes per cow). In these systems, high genetic merit cows are able to efficiently produce high quality milk, as feed energy supply will be optimised by both the range of energy-rich forages and the higher proportion of concentrates that can be included in the diet, leading to both the magnitude and length of the post-calving negative energy balance being minimised (Knaus *et al*., 2001; Weller and Bowling, 2004).

Within some European countries with larger-scale dairy systems, organic milk production has been dominated by breeds that have been selected and bred for more intensive conventional systems in which a higher proportion of concentrates is included in the annual diet. For example, in both the Netherlands and the UK the high genetic merit Holstein breed is either the sole or the main breed in the majority of organic dairy herds. However, while the Holstein has performed well in systems feeding a higher proportion of concentrates in the diet, the potential benefits from other breeds and also crossbreds are currently making an increasing contribution to the total production of organic milk in less intensive systems, including ensuring that milk quality components are maintained at a satisfactory level throughout the year, and that the productive life (i.e. longevity) of cows within the herd is increased due to improved reproductive status. For example, in Switzerland successful breeding programmes have been achieved with both the Brown Swiss and the Swiss Red and White breeds to improve the compatibility between genetics and the system of management (Bapst, 2001). In the UK the potential benefits of cows with a less demanding nutrient requirement, including the Ayrshire, Guernsey, Jersey and Shorthorn traditional breeds, are now widely recognised.

However, when comparing the suitability of dairy breeds with different genetic merit values for organic systems, it is important to note that in a study comparing the performance of high genetic merit cows in both low and high concentrate input systems (Weller and Bowling, 2004), there were marked differences in performance between individual cows in both systems. For

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

example, the majority of cows maintained satisfactory yields, milk quality and reproductive efficiency when fed <0.5 tonnes of concentrates per lactation, but metabolic disorders, a high milk fat to protein ratio and delayed conception also occurred with some cows due to a feed energy deficit. It is important to note that despite the differences found between the Holstein–Friesian cows in the above study, genetic variation is likely to be greater between breeds than within breeds.

Differences between breeds may be important within self-sufficient organic systems, as the diets are potentially more vulnerable to mineral deficiencies that can affect the health status and performance of the organic cow. For example, Bakke (2003) reported that Jerseys, Norwegian Reds and Swedish Red breeds are more prone to milk fever, and in the USA farmers are advised to include extra calcium in the diets of Jersey cows. Roderick and Hovi (1999) also reported a higher incidence of milk fever in coloured breeds compared to black and white cows. Jerseys are also susceptible to copper toxicity, which is a problem when high molybdenum concentrations occur in forages grown under stressed conditions, e.g. periods of low rainfall and slow rates of plant growth.

Optimal lifetime efficiency has been reported as being achieved when cows have a productive life of six lactations (Webster, 2000; Moorby *et al*., 2003), attributable to lower culling rates and a better ratio of productive to non-productive cattle due to the requirement for fewer replacements. Longevity is an important factor in organic dairy herds; it has both benefits and disadvantages, including the influence on milk quality, and is often attributed to the differences between dairy breeds. Benefits of longevity include improved financial margins per hectare and improved welfare standards associated with the cow having a longer productive life, while the disadvantages include lower immunity status, a higher risk of disease with increasing age and reduced milk quality, particularly high somatic cell counts, lower total protein and a decrease in the casein to non-casein ratio. The somatic cell counts in the milk of older cows within an organic herd are markedly higher than those in younger cows (Weller and Davies, 1998). Therefore, while the aim may be to extend the productive life of cows, in some herds older cows have to be culled to keep the average somatic cell count at a level that meets acceptable standards for both the liquid and processing markets.

IP Address: 129.132.208.100

## 11.4 Future trends that may influence the quality of organic milk

Concerns have been expressed that the increases in the size of dairy units that are occurring in both conventional and organic systems in some countries may have an adverse effect on welfare standards and milk quality. However, increasing herd size is an inevitable change for many countries and cannot be classified as either beneficial or detrimental, as the production of quality organic milk from well-managed herds with a good health status is dependent on the standards of management and husbandry, not on the number of cows *per se*. For

© Woodhead Publishing Limited, 2010

example, the production of high quality milk is achieved successfully in Danish organic herds even though many of the farms operate large organic units.

The minimum standards defined for the quality of milk produced on dairy farms have been raised in recent years. Further changes, including the requirement to reduce the average herd somatic cell count, will not be of concern to the majority of organic farmers. However, for others any reduction of cell count will require investigation of the causes of high cell counts, and will necessitate either an improvement in the current management and husbandry practices or the culling of a higher proportion of the herd, due to sub-clinical infections causing the high cell counts of individual cows.

The increasing demand in the future for nutrients, fossil fuels and water for the production of milk and other food products will increase the need to produce quality milk from more sustainable systems, and the challenge will be to grow high quality forages within the farm system and to utilise them more efficiently. The greatest changes are likely to occur where significant quantities of feed need to be purchased to maintain a more intensive system, with the increasing demands for grain crops for human consumption, biofuels and monogastric animals also likely to increase the cost of purchased concentrate feeds to an unacceptable level.

Future climatic changes will also influence the type of crops that are grown on organic dairy farms if a viable level of high quality milk is to be maintained. Areas with increasing rainfall and milder winter temperatures are likely to benefit from an enhanced performance from the grass and white clover swards. This will have a positive effect on milk quality due to an extended grazing season and a greater proportion of fresh herbage in the annual diet. However, areas experiencing reduced annual rainfall will need to maintain the current annual quantity and quality of herbage by growing alternative crops to compensate for the lower production from the grass and white clover swards, including growing deeper-rooted crops that include chicory and lucerne. Replacing spring-sown with autumn-sown crops that reach acceptable yields prior to low summer rainfall periods, including whole-crop cereals and grasses with early spring growth, will also have an important role in the cropping strategy.

IP Address: 129.132.208.100

## 11.5 References

AL-MABRUK R M, BECK N F G and DEWHURST R J (2004), 'Effects of silage species and supplemental vitamin E on the oxidative stability of milk', *Journal of Dairy Science*, **87**, 406–412.

AULDIST M J, COATS S, SUTHERLAND B J, MAYES J J, MCDOWELL G H and ROGERS G L (1996), 'Effects of somatic cell count and stage of lactation on raw milk composition and the yield and quality of Cheddar cheese', *Journal of Dairy Research*, **63**, 269–280.

BAKKE M J (2003), 'Feeding and management of Jerseys and Holsteins: should there be a difference?', in Garnsworthy P C and Wiseman J, *Recent Advances in Animal Nutrition*, Nottingham University Press, Nottingham, UK.

© Woodhead Publishing Limited, 2010

BAPST B (2001), 'Swiss experiences on practical cattle breeding strategies for organic dairy herds', in Hovi M and Baars T, *Proceedings of the 4th NAHWO Workshop*, 2001, Wageningen, The Netherlands, University of Reading, Reading, UK.

BEEVER D E, CAMMELL S B, SUTTON J D, ROWE N and PERROTT G E (1998), 'Energy balance in high-yielding cows', *Proceedings of the British Society of Animal Science*, 1998, Scarborough, UK.

BELL J F, OFFER N W and THOMAS Z (2006), 'The effect of grazing management on levels of conjugated linoleic acid in bovine milk', *Proceedings of the British Grassland Research Conference*, 2006, Cirencester, UK.

BLOWEY R and EDMONDSON P (1995), *Mastitis Control in Dairy Herds*, Farming Press Books, Ipswich, UK.

BOSBERRY S and DOBSON H (1989), 'Periparturient diseases and their effect on reproductive performance in five dairy herds', *Veterinary Record*, **124**, 217–219.

BUTLER W R, CALAMAN J J and BEAM S W (1996), 'Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle', *Journal of Animal Science*, **74**, 858–865.

CHAMBERLAIN A T and WILKINSON J M (1996), *Feeding the Dairy Cow*, Chalcombe Publications, Lincoln, UK.

CHARRIER X, EMILE J C and GUY P (1993), 'Recherche de génotypes de luzerne adaptes au pasturage', *Fourrages*, **135**, 507–510.

DHIMAN T R, ANAND G R, SATTER L D and PARIZA M W (1999), 'Conjugated linoleic acid content of milk from cows fed different diets', *Journal of Dairy Science*, **82**, 2146–2156.

DUFFIELD T F, KELTON D F, LESLIE K E, LISSEMORE K D and LUMSDEN J H (1997), 'Use of test day milk fat and milk protein to detect sub-clinical ketosis in dairy cattle in Ontario', *Canadian Veterinary Journal*, **38**, 713–718.

ELLIS K A, INNOCENT G T, MIHM M, CRIPPS P, MCLEAN W G, HOWARD C V and GROVE-WHITE D (2007), 'Dairy cow cleanliness and milk quality on organic and conventional farms in the UK', *Journal of Dairy Research*, **74**, 302–310.

EMERY R S (1978), 'Feeding for increased milk protein', *Journal of Dairy Science*, **61**, 825–828.

GOFF J P and HORST R L (1997), 'Physiological changes at parturition and their relationship to metabolic disorders', *Journal of Dairy Science*, **80**, 1260–1268.

GORDON F J, PATTERSON D C, YAN T, PORTER M G, MAYNE C S and UNSWORTH E F (1995), 'The influence of genetic index for milk production on the response to complete diet feeding and the utilisation of energy and nitrogen', *Animal Science*, **61**, 199–210.

HEUER C, VAN STRAALEN W M, SCHUKKEN Y H, DIRKZWAGER A and NOORDHUIZEN J P T M (2000), 'Prediction of energy balance in a high yielding dairy herd in early lactation: model development and precision', *Livestock Production Science*, **65**, 91–105.

KLEI L, YUN J, SAPRU A, LYNCH J, BARBANO D, SEARS P and GALTON D (1998), 'Effects of milk somatic cell count on cottage cheese yield and quality', *Journal of Dairy Science*, **81**,1205–1213.

KNAUS W F, STEINWIDDER A and ZOLLITSCH W (2001), 'Energy and protein balances in organic dairy cow nutrition – model calculations based on EU regulations', in Hovi M and Baars T, *Proceedings of the 4th NAHWO Workshop*, 2001, Wageningen, The Netherlands, University of Reading, Reading, UK.

KOLVER E S and MULLER L D (1998), 'Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration', *Journal of Dairy Science*, **81**, 1403–1411.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

KREUZER M, VON SIEBENTHAL A M, KAUFMANN A, RATZER H, JAKOB E and SUTTER F (1996), 'Determination of the relative efficacy to enhance milk renneting properties of alterations in the dietary energy, breed and stage of lactation', *Milchwissenschaft*, **51**, 633–637.

KRUTZINNA C, BOEHNCKE E and HERMANN H J (1996), 'Organic milk production in Germany', *Biological Agriculture and Horticulture*, **13**, 351–358.

MA Y, RYAN C, BARBANO D M, GALTON D M, RUDAN M A and BOOR K J (2000), 'Effects of somatic cell count on quality and shelf-life of pasteurized fluid milk', *Journal of Dairy Science*, **83**, 264–274.

MANSON F J and LEAVER J D (1988), 'The influence of dietary protein intake and of foot trimming on lameness in dairy cattle', *Animal Production*, **47**, 191–199.

MAYNE C S and PEYRAUD J L (1996), 'Recent advances in grassland utilisation under grazing and conservation', *Grassland and Land Use Systems, Proceedings of the 16th EGF meeting, Volume 1*, 347–360.

MOORBY J M, ESSLEMONT R J and JONES R (2003), 'A review of the effect of dairy breed and cross-breeding on fertility, health and longevity', *Milk Development Council Report IS0213*, Milk Development Council, Cirencester, UK.

NAUTA W J, BAARS T and BOVENHUIS H (2006), 'Converting to organic dairy farming: consequences for production, somatic cell scores and calving interval of first parity Holstein cows', *Livestock Science*, **99**, 185–195.

OFFERHAUS E J, BAARS T and GROMMERS F J (1994), *Gezondheid en vruchtbaarheid van melkvee op biologische bedrijven*, Report by the Louis Bolk Institute, Driebergen, The Netherlands.

PHILLIPS C J C (1988), 'The use of conserved forage as a supplement for grazing dairy cows', *Grass and Forage Science*, **43**, 215–230.

PHIPPS R H, SUTTON J D and JONES B A (1995), 'Forage mixtures for dairy cows; the effect on dry matter intake and milk production of incorporating either fermented or urea-treated whole crop wheat, brewers grains, fodder beet or maize silage into diets based on grass silage', *Animal Science*, **61**, 491–496.

PLYM FORSHELL K (1994), 'Metabolic profiles in milk', *Cattle Practice*, **2**, 525–536.

RENAU J K (1986), 'Effective use of dairy herd improvement somatic cell counts in mastitis control', *Journal of Dairy Science*, **69**, 1708–1720.

RODERICK S and HOVI M (1999), 'Animal health and welfare in organic livestock systems: Identification of constraints and priorities', *MAFF Report for Project OF0172*, MAFF, London, UK.

ROEPSTAD E, LARSEN H J and REFSDAL A O (1989), 'Immune function in dairy cows related to negative energy balance and metabolic status in early lactation', *Acta Veterinaria Scandinavica*, **30**, 209–219.

RYAN G, MURPHY J J, CROSSE S and RATH M (2003), 'The effect of pre-calving diet on post-calving cow performance', *Livestock Production Science*, **79**, 61–71.

SPORNDLY E (1989), 'Effects of diet on milk composition and yield of dairy cows with special emphasis on milk protein content', *Swedish Journal of Agricultural Research*, **19**, 99–106.

THOMAS C, LEACH K A, LOGUE D.N, FERRIS C and PHIPPS R H (1999), 'Management options to reduce load', in *Metabolic Stress in Dairy Cows. Occasional Publication 2,* British Society of Animal Science, 129–139.

THUEN E, STEINSHAMM U T, BRENOE U T, YRI C and EKERHOLT G (2002), 'Effect of concentrate level on forage intake, milk production and energy and nitrogen utilisation in organic milk production', in Kyriazakis I and Zervas G, *Organic Milk*

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

*and Meat from Ruminants*, EAAP Publication 106, Wageningen Academic Publishers, Wageningen, The Netherlands.

URECH E, PUHAN Z and SCHALLIBAUM M (1999), 'Changes in milk protein fraction as affected by subclinical mastitis', *Journal of Dairy Science*, **82**, 2402–2411.

VAARST M and ENEVOLDSEN C (1994), 'Disease control and health in Danish organic dairy herds – biological basis of sustainable animal production', in Huisman E A, Osse J W M, van der Heide D, Tamminga S, Tolkamp B J, Schouten W G P, Hollingworth C E and van Winkel G L, *Proceedings of the 4th Zodiac Symposium*, EAAP Publication 64, Wageningen Academic Publishers, Wageningen, The Netherlands.

VILLA-GODOY A, HUGHES T L, EMERY R S, CHAPLIN T L and FOGWELL R L (1988), 'Association between energy balance and luteal function in lactating dairy cows', *Journal of Dairy Science*, **71**, 1063.

WEBER S, PABST K, SCHULTE-COERNE H, WESTPHAL R and GRAVERT H O (1993), 'Five year studies on conversion to organic milk production, 1, Production technology', *Zuchtungskunde*, **65**, 325–337.

WEBSTER A J F (2000), 'Sustaining fitness and welfare in the dairy cow', *Proceedings of the New Zealand Society of Animal Production*, **60**, 207–213.

WELLER R F and BOWLING P J (2000), 'Health status of dairy herds in organic farming', *Veterinary Record*, **146**, 80–81.

WELLER R F and BOWLING P J (2004), 'The performance and nutrient use efficiency of two contrasting systems of organic milk production', *Biological Agriculture and Horticulture*, **22**, 261–270.

WELLER R F and COOPER A (2001), 'Seasonal changes in crude protein concentration of white clover/perennial ryegrass swards grown without fertiliser N in an organic farming system', *Grass and Forage Science*, **56**, 92–95.

WELLER R F and DAVIES D W R (1998), 'Somatic cell counts and incidence of clinical mastitis in organic milk production', *Veterinary Record*, **143**, 365–366.

ZOLLITSCH W, KRISTENSEN T, KRUTZINNA C, MACNAEIHDE F and YOUNIE D (2004), 'Feeding for health and welfare: the challenge of formulating well-balanced rations in organic livestock production', in Vaarst V, Roderick S, Lund V and Lockeretz W (eds), *Animal Health and Welfare in Organic Agriculture*, CABI Publishing, Wallingford, UK.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

# 12

# Improving goat milk

**Y. Park, Fort Valley State University, USA**

**Abstract:** Production of goat milk is of great importance not only for underdeveloped countries, where it provides basic nutrition and subsistence to rural people, but also for developed countries as a valued part of the total dairy industry, where it provides diversity to sophisticated consumer tastes, and supports people with allergies and gastro-intestinal disorders. High quality goat milk can be produced, and great progress has been made to improve the acceptability of dairy goat products by consumers. Properly produced goat milk has no objectionable flavor, is free of spoilage bacteria, and contains the legal minimum limits of all nutrients.

**Key words:** goat milk production, factors affecting quality, improving production techniques, genetic polymorphisms, processing technology.

IP Address: 129.132.208.100

## 12.1 Introduction: key issues in improving goat milk

Goat milk has played a major role in the economic viability of many developing countries of the world, as well as some developed countries in the Mediterranean region, including France, Italy, Spain, and Greece, through its utilization for manufacture of cheeses and other products (Juàrez and Ramos, 1986; Kosikowski, 1986; Park and Guo, 2006). In addition to the economic and nutritional significance of goat milk in many developing countries, goat milk products also have recently gained increasing popularity among milk allergy patients, healthfood lovers, gourmet lovers, goat farmers and cheese enthusiasts in the US and other developed countries (Kosikowski, 1986; Park, 1990).

On the other hand, the goat has been regarded as the most maligned domesticated animal in many parts of the world due to its sometimes offensive odor (Rubino and Claps, 1995; Haenlein, 2006). The odor, especially from the

© Woodhead Publishing Limited, 2010

buck, floats strongly around the premises and can affect the flavor of the doe's milk if the ventilation, milking procedures and cooling of the milk are not properly managed on the goat farm (Haenlein, 2006). This buck odor is an aphrodisiac for the doe, enticing her libido, and is part of the 'buck effect' to stimulate sexual activity (Mellado *et al.*, 2000; Ott *et al.*, 1980). However, it has been demonstrated in recent years that properly milked and cooled goat milk is odor free and hard to distinguish from cow milk in odor and taste (Campbell and Marshall, 1975; Mowlem, 1988). Therefore, production of quality goat milk is possible, and great progress has been made in recent years to debunk this old prejudice against goat milk by consumers (Haenlein, 2006).

Production of goat milk is highly important not only for underdeveloped countries, where it provides basic nutrition and subsistence to rural people, but also for developed countries as a valued part of the total dairy industry, where it provides diversity to sophisticated consumer tastes, and supports people with medical afflictions, such as allergies and gastro-intestinal disorders (Haenlein, 1986, 1996, 1997; Park, 1992, 1994a,b). Thus, goat milk serves, in a general way, three types of markets around the world: (a) home consumption, (b) specialty gourmet interests, and (c) medical needs (Park and Haenlein, 2007).

Producing high quality raw milk is of paramount importance for successful production and marketing of dairy goat products. They must be safe to consume, and free of pathogenic bacteria, antibiotic, insecticide and herbicide compounds. They should have good and no objectionable flavor, be free of spoilage bacteria, and contain legal minimum limits of all nutrients (Loewenstein *et al.*, 1984; Park and Guo, 2006). The purpose of this chapter is to address the key issues involved in improving the safety and quality of goat milk production and processing for human consumption.

IP Address: 129.132.208.100

## 12.2 Production of quality goat milk

### 12.2.1 General principles

Production of high quality goat milk, or any other milk, largely depends on the farm producer as well as workers at dairy processing plants. Quality goat milk production should start at the farm level, because the flavor and quality of the milk cannot be improved later in the processing stage (Park and Guo, 2006). The general principle is that the better the raw milk, the better the processed products.

Raw milk produced from the lacteal glands is highly perishable, and its quality is easily affected by many factors such as feeding, handling of animals prior and during milking, handling of the milk during and after milking, cooling, transportation, pasteurization, processing, packaging, processing utensils, etc. (Peters, 1990; Haenlein, 1992). Since milk is a highly nutritious medium for bacterial growth, it is liable to deteriorate rapidly. A clean milking environment is just as important as the milk composition. Good quality milk must contain no harmful pathogens or microorganisms likely to damage the cultured dairy

© Woodhead Publishing Limited, 2010

products, nor such foreign substances as antibiotics, antiseptics or pesticide residues (Kosikowski, 1977; Loennerdal *et al.*, 1981; Le Jaouen, 1987). In addition, basic bacterial flora should not be too numerous.

In commercial milk production channels, at least five major parameters are routinely monitored by various regulatory agencies in order to safeguard quality milk production. These are (i) nutritional constituents in milk, (ii) somatic cell counts as related to mastitis, (iii) bacteria counts as related to sanitary practices, (iv) adulteration and pesticide residue contents, and (v) flavor, taste, appearance and temperature (Haenlein, 1992; Park and Guo, 2006).

Off-flavor in goat milk can be attributed to feeds, weeds, forages, chemicals, building materials, colostrum, estrus, mastitic milk, filthy utensils and strainer, unclean milking equipment, slow cooling, and odors from bucks, barn and/or milk room. Feeding odorous feeds at least two hours before feeding is not recommended (Park and Guo, 2006).

The 'goaty' flavor can be prevented by good management, healthy lactating does, and sanitary milking procedures and a good ventilation system in the milking parlor. Rancidity and goaty flavor can also be avoided by minimizing disruption of the milk fat globule membrane and its exposure to lipase enzyme during processing. The short-chain free fatty acids, capric, caproic and caprylic acids, generated by the lipase in goat milk are considered to cause the goaty and rancid tastes (Park and Guo, 2006).

It cannot be overstated that good quality milk requires good management of the entire farm system. This can be achieved by following the recommended daily milking practices, maintaining functioning and sanitary equipment, keeping healthy animals, and using recommended detergent, acid and sanitizers for cleaning and milking equipment.

IP Address: 129.132.208.100

### 12.2.2 Regulatory standards for quality goat milk and its products

Goats secrete milk through the apocrine process, while cows produce milk by the merocrine process. Even though goat milk contains naturally higher SCCs (somatic cell counts) than cow milk due to the apocrine secretory process, the same regulations for milk quality standards are enforced for both species. It is common to find high SCC in goat milk when actual numbers of leucocytes are relatively low (Kapture, 1980; Park and Humphrey, 1986). Dairy goat producers on the National Conference on Interstate Milk Shipments have very actively pursued this problem of SCC legal thresholds (Kapture, 1982; Haenlein, 1992).

For measuring raw milk quality, SCC has been accepted as a quantitative index for mastitic conditions or degree of glandular irritation in the mammary gland (Park and Humphrey, 1986). Milk with high somatic cells and spoilage bacteria results in poor quality products. SCC can be determined by various tests including the California Mastitis Test and Wisconsin Mastitis Test. The regulations for all aspects of production, processing, and marketing milk in the US are described in the Grade A Pasteurized Milk Ordinance (PMO) published by the Food and Drug Administration (FDA). From these standards for Grade A

© Woodhead Publishing Limited, 2010

milk, each state health department establishes its minimum regulations (Peters, 1990). In the European Union, similar standards are enforced such as Codex or IDF (International Dairy Federation) regulations. Some states in the US may adopt more stringent standards than the PMO regulations. The state of Oregon, for example, has set its somatic cell count (SCC) standard at 750,000 cells per ml, while the PMO standard is one million per ml. The state of Georgia has recently raised the SCC for goat milk to 1,000,000 cells per ml as the maximum cell count.

Grade A goat milk has at least four important requirements for quality. These are (i) safe to drink, (ii) good flavor, (iii) relatively free from spoilage bacteria and somatic or body cells, and (iv) composition (Loewenstein *et al.*, 1984). Pasteurization is the most important process to remove pathogens for assurance of safe milk, though it does not remove chemical contaminants. Good flavor of goat milk comes from a clean, healthy, properly managed goat herd, where the ideal flavor is slightly sweet and slightly salty with complete absence of strong odors and flavors. Oxidized flavor is caused by nutritional imbalances or exposure to light. Rancid or goaty flavor develops when the fat is partially disintegrated by enzyme action. These off-flavors can be controlled by pasteurization and protection of the milk from sun and UV light (Park and Guo, 2006).

All Grade A pasteurized milk and milk products must be produced, processed, and pasteurized to conform with the specific PMO codes. For fluid milk, standard milk composition refers to the levels of major nutrients such as fat, protein, lactose, and minerals. The Public Health Service, FDA defines milk to contain a minimum of 3.25% fat and 8.25% not-fat milk solids, which is the sum of the protein, lactose, and mineral content. Even though the FDA standards have been generated based on cow milk, the same definition and regulations have been applied to goat milk (Park and Guo, 2006).

IP Address: 129.132.208.100

An example of regulations for the chemical, bacteriological, and temperature standards, and the sanitation requirements, is presented in Table 12.1 (Colorado Department of Health, 1980). The quality control guidelines for microbiological standards in dairy foods are shown in Table 12.2 (Guthrie, 1983). Milk qualities are also determined by physico-chemical and enzymatic indices as well as specific milk components, including specific gravity, freezing point, SH value, titratable acidity, redox potential, electrical conductivity, and enzyme levels (i.e., alkaline phosphatases, lipoprotein lipases and proteinases) of the milk.

### 12.2.3 Essential control systems for production of quality dairy goat products

In order to have control systems for production of quality milk and dairy products, a Dairy HACCP Safety System is recommended to be established for each commercial dairy plant or individual dairy goat processing facility. The IDFA (International Dairy Foods Association, Washington, DC) recommends the prerequisite programs to be effectively monitored and controlled before developing an HACCP plan. The comprehensive, effective prerequisite programs will simplify HACCP plans and will ensure that the integrity of the

© Woodhead Publishing Limited, 2010

**Table 12.1** Chemical, bacteriological and temperature standards

| | |
|---|---|
| *Grade A raw milk for pasteurization* | |
| Temperature | Cooled to 45ºF (7ºC) or less within two hours after milking, provided that the blend temperature after the first and subsequent milkings does not exceed 50ºF (10ºC). |
| Bacterial limits | Individual producer milk not to exceed 100,000 per ml prior to commingling with other producer milk. Not to exceed 300,000 per ml as commingled milk prior to pasteurization. |
| Antibiotics | Individual producer milk: No detectable zone with the *Bacillus subtilis* method or equivalent. Commingled milk: No detectable zone by the *Sarcina lutea* Cylinder Plate Method or equivalent. |
| Somatic cell count | Individual producer milk not to exceed 1,500,000 per ml. |
| *Grade A pasteurized milk and milk products* | |
| Temperature | Cooled to 45ºF (7ºC) or less and maintained thereat. |
| Bacterial limits | 20,000 per ml.[a] |
| Coliforms | Not to exceed 10 per ml, provided that, in the case of bulk milk transport tank shipments, shall not exceed 100 per ml. |
| Phosphatase | Less than 1 microgram per ml by the Scharer Rapid Method or equivalent. |
| Antibiotics | No detectable zone by the *Sarcina lutea* Cylinder Plate Method or equivalent. |

[a] Not applicable to cultured products.
Source: Data from Colorado Department of Health (1980), *Colorado Grade A Pasteurized Fluid Milk and Milk Products Regulations*, Denver, CO; also adapted from Park and Guo (2006).

IP Address: 129.132.208.100

HACCP processing plant is maintained and that the manufactured product is safe. The prerequisite programs are described in Table 12.3.

Table 12.3 lists the seven different essential prerequisite areas for developing a comprehensive HACCP plan for a commercial dairy plant. These areas can also be utilized as the reference guidelines of a quality control program for a small, dairy goat farm operation. There are two important areas that require special attention:

1. For outside property, the land ought to be free of debris and refuse, and should not be in close proximity to any source of pollution (e.g. objectionable odors, smoke, dust or other contaminants). Roadways are properly graded, compacted, dustproof, and drained. Premises and shipping and receiving areas provide or permit good drainage.
2. For the infrastructure, the building and facilities are designed to readily permit cleaning, and prevent entrance, harboring of pests and entry of environmental contaminants (IDFA, 1998).

Hygienic practices are integral components for the production of quality milk and dairy products. Ongoing training in personal hygiene and hygienic handling

© Woodhead Publishing Limited, 2010

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

**Table 12.2** Quality control guidelines for microbiological standards in dairy foods

| Product | Standard plate count | Coliforms | Psychrotrophic SPC after 5 d at 70ºF | Yeast and mold | Staphylococci | Salmonella |
|---|---|---|---|---|---|---|
| Raw milk – bulk tankers | <1000–50,000 | <100–<1000 | <10,000–<100,000 | – | – | – |
| Commingled raw milk at pasteurizer | <50,000–30,000 | <100–<1000 | 100,000–<800,000 | – | <5000–<100,000 | – |
| Pasteurized Grade A fluid products | <1000–<10,000 | <1–<5 | <20,000–<69,000 | – | <1 | <1 |
| Ice cream | <20,000–50,000 | <1–<10 | <50 | | <1 | <1 |
| Cottage cheese (dry) | <1000–20,000 | <1–<5 | <10,000–<100,000 | <5–<10 | <1 | <1 |
| Butter | <5000–<20,000 | | <50,000 | <5–<10 | <1 | <1 |
| Milk powder | <20,000–<50,000 | NS | NS | <10 | <1 | <1 |

Source: adapted from Guthrie (1983).

**Table 12.3** Prerequisite areas of developing an HACCP plan

| | |
|---|---|
| 1. Premises | |
| a. Outside property | b. Building |
| c. Sanitary facilities | d. Water quality program |
| 2. Receiving/storage/shipping | |
| a. Receipt of raw materials, ingredients, and packaging materials | |
| b. Specifications | c. Storage |
| d. Distribution | |
| 3. Equipment performance and maintenance | |
| a. General equipment design | b. Equipment installation |
| c. Equipment maintenance | |
| 4. Personnel training program | |
| a. Manufacturing control | b. Hygienic practices |
| c. Controlled access | d. Personnel safety |
| 5. Cleaning and sanitation | |
| a. Cleaning and sanitation program | b. Pest control program |
| 6. Recall programs | |
| a. Traceability | b. Recall system |
| c. Recall initiation | |
| 7. Supplier control programs | |
| a. Performance criteria | b. Alternative sources |

Source: IDFA (1998).

of food should be provided to all persons and food handlers entering food handling premises. The major areas of hygienic practices include (i) communicable diseases, (ii) injuries, (iii) washing of hands, (iv) personal cleanliness and conduct, (v) controlled access, and (vi) personal safety, etc. (IDFA, 1998).

IP Address: 129.132.208.100

### 12.2.4 HACCP plans and hazard components in production of quality dairy products

For the production of quality Grade A milk and its manufactured products, HACCP plans can be initiated for better control systems at a milk processing plant. Seven different principles must be implemented. These are (i) conduct a hazard analysis, (ii) identify critical control points, (iii) establish critical limits for each critical control point, (iv) establish monitoring procedures, (v) establish corrective actions, (vi) establish recordkeeping procedures, and (vii) establish verification procedures (Park and Guo, 2006). The HACCP plans for milk and different manufactured products can be implemented at different locations with some modifications depending on specific situations of individual processing plants.

Hazards associated with production of milk and dairy products are classified in three different areas as microbiological, chemical, or physical hazards. Tables 12.4 and 12.5 depict the hazards as listed by the National Advisory Committee on Microbiological Criteria for Foods (IDFA, 1998). The sources of the hazard components involved in several milk products are exemplified by possible sources as shown in Table 12.6.

© Woodhead Publishing Limited, 2010

**Table 12.4** Microbiological hazards for dairy products

| Severe | Moderate with potentially extensive spread | Moderate with limited spread |
|---|---|---|
| *Brucella* | *Salmonella* spp. | *Bacillus cereus* |
| *Clostridium botulinum* | Enterotoxigenic bacteria | *Campylobacter jejuni* and other species |
| *Listeria monocytogenes* | *Escherichia coli* | |
| *Salmonella typhi*, *paratyphi*, and *dublin* | Enteroinvasive *Escherichia coli* | *Clostridium perfringens* |
| *Shigella dysenteriae* | *Shigella* spp. | *Staphylococcus aureus* |
| Hepatitis A and E | Viruses | *Aeromonas* spp. |
| *Escherichia coli* O157:H7 | *Cryptosporidium* protozoa | *Yersinia enterocolitica* |
| | | Parasites |

Source: IDFA (1998).

**Table 12.5** Chemical and physical hazards for dairy products

| Chemical hazards | | Physical hazards |
|---|---|---|
| Natural toxins | *Mycotoxins*<br>• Acute: ochratoxin, trichothecene, zearalenone, aflatoxin<br>• Chronic: aflatoxin, sterigmatocystin, patulin<br>*Other natural*<br>Thyro-toxicosis | Metal<br>Glass<br>Insect/pest parts<br>Dirt<br>Wood fragments<br>Personal effects<br>Plastic<br>Others |
| Metals | Copper, cadmium, mercury | |
| Drug residues | Beta-lactams, sulfonamides, tetracyclines, others | |
| Sanitizer residues | Chlorinated, fatty acid, iodophors, others | |
| Pesticide residues | | |
| Allergens | Peanuts, tree nuts | |
| Food additives | | |
| Inadvertent chemicals | Lubricants, boiler additives | |

Source: IDFA (1998).

IP Address: 129.132.208.100

In an effort to ensure quality goat milk and cheese production, different types of HACCP plans can be practiced at different goat dairies. The flowcharts for manufacturing fluid goat milk and Monterey Jack goat cheese at Fort Valley State University showing several critical control points are illustrated in Figs 12.1 and 12.2. The general flow diagram of fluid milk processing for the HACCP plans recommended by IDFA is shown in Fig. 12.3. However, each individual dairy manufacturer may adapt and/or modify the HACCP plans for their own plant control systems depending on their specific plant locations, resource availabilities and peculiarities.

© Woodhead Publishing Limited, 2010

**Table 12.6** Sources of hazards for different milk products

| | | |
|---|---|---|
| **A. Raw milk** | | |
| Microbiological: | | |
| *Salmonella* | *B. cereus* | *E. coli* |
| *S. aureus* | *Brucella* | *Campylobacter* |
| *C. perfringens* | *L. monocytogenes* | *Shigella* |
| *Yersinia* | Staphylococcal enterotoxin | |
| Chemical: | | |
| Antibiotics | Pesticides | Sulfonamides |
| Physical: | | |
| Insects | Soil | Glass fragments |
| **B. Cheese** | | |
| Microbiological: | | |
| *L. monocytogenes* | *Campylobacter* | *Shigella* |
| *Brucella* | *Salmonella* | *Clostridium botulinum* |
| *S. aureus* | Staphylococcal enterotoxin | |
| Chemical: | | |
| Nitrates, nitrites | Pestidices | Aflatoxins |
| **C. Dried milk products:** | | |
| Microbiological: | | |
| *Salmonella* | *C. perfringens* | *E. coli* |
| Staphylococcal enterotoxin | *S. aureus* | *C. botulinum* |
| *L. monocytogenes* | | |
| Chemical: | | |
| Sulfonamides | Pesticides | Antibiotics |

Source: IDFA (1998).

IP Address: 129.132.208.100

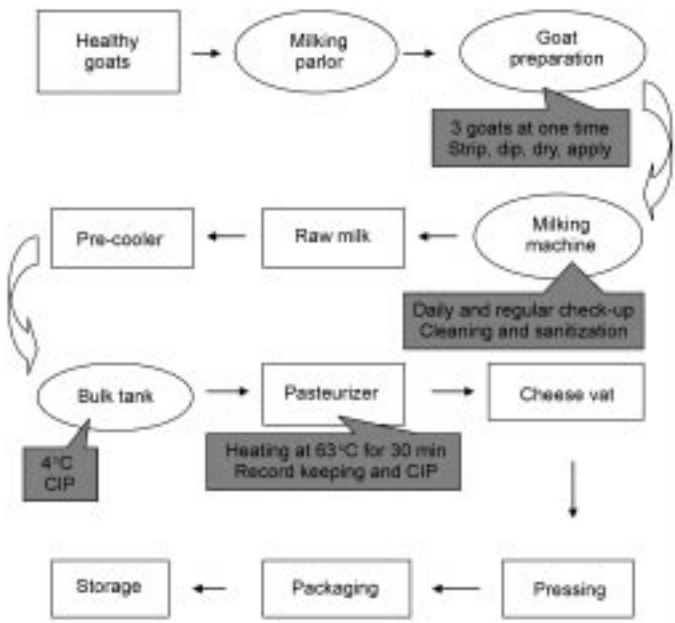
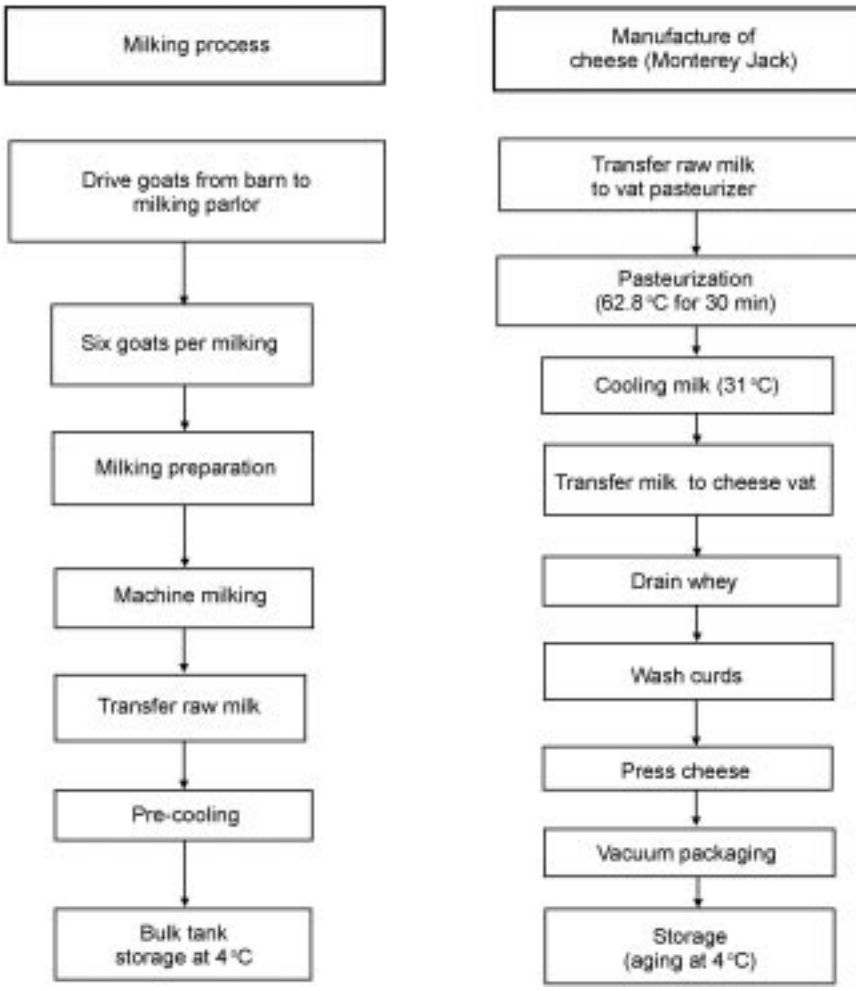



**Fig. 12.1** HACCP: Flow diagram of processing (Fort Valley State University, Georgia, USA) (Park and Guo, 2006).

© Woodhead Publishing Limited, 2010




IP Address: 129.132.208.100

**Fig. 12.2** Flow diagram for milking and cheese manufacture processes (Fort Valley State University, Georgia, USA) (Park and Guo, 2006).

### 12.2.5 Guidelines for pasteurization and prevention of post-pasteurization contamination

Pasteurization is the critical step for killing harmful pathogenic organisms in the raw milk. Controlling cross-contamination before, during and after pasteurization is also extremely important for ensuring food safety of the processed milk products. As a cooperative effort between the Food and Drug Administration and the International Dairy Foods Association, the guidelines associated with pasteurization and post-pasteurization contamination were originally issued during September 1986, revised in 1987, and again revised by IDFA (1998). The IDFA (1998) has suggested the following guidelines on pasteurization, vat pasteurization and post-pasteurization contamination.

© Woodhead Publishing Limited, 2010

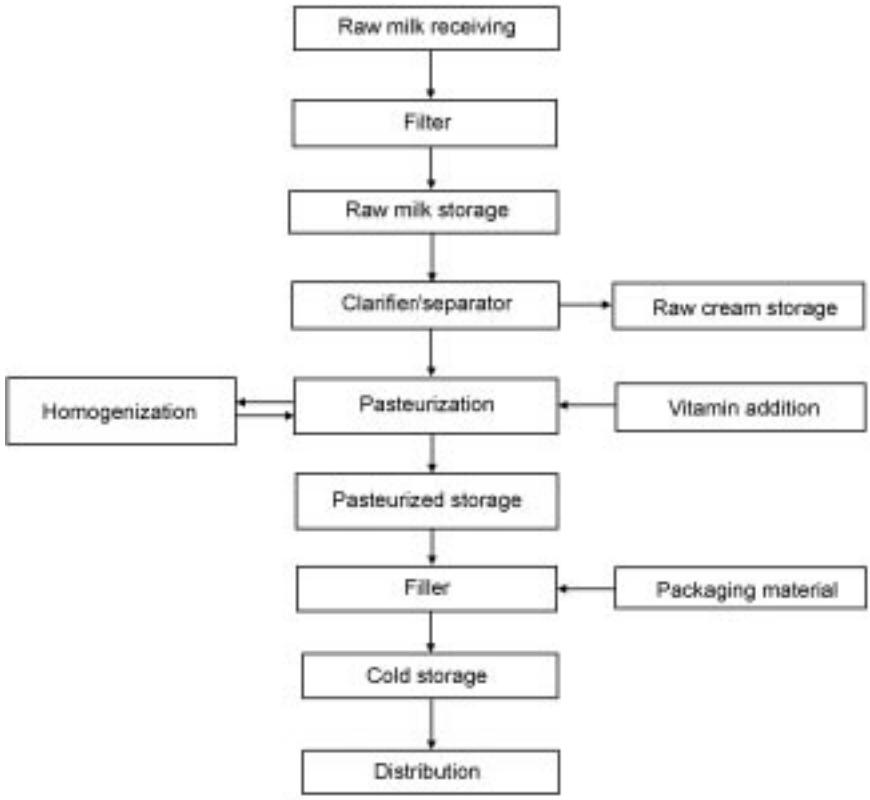



**Fig. 12.3** General flowchart for commercial fluid milk processing procedures (adapted from FDA Workshop, St Louis, MO, 2000).

IP Address: 129.132.208.100

*Pasteurization*

The PMO code for the basic pasteurization principle is that 'every particle of milk or milk product be heated to at least a minimum temperature and held at that temperature for at least the specified time in properly designed and operated equipment.' Every plant should assess the adequacy of their pasteurization equipment to determine if it satisfies the basic principle of pasteurization. It is also necessary that dairy products containing higher fat and/or added sugars or which are viscous (e.g. frozen dessert mixes, cream, eggnog, etc.) also require higher pasteurization temperatures and/or longer times. The standard time and temperature pasteurization conditions for milk and high-solid dairy products are shown in Table 12.7.

For all HTST pasteurizing systems, a properly designed, installed, and operating flow diversion device and properly operating pressure controls for regenerator systems are required to be installed. It is also recommended that all Grade A products as well as frozen dessert mixes must be pasteurized in the plant of final processing and packaging. The heat exchanger (presses) of HTST

© Woodhead Publishing Limited, 2010

**Table 12.7** Minimum pasteurization temperatures and times recognized by the US Public Health Service and Food and Drug Administration

| Product | Temperature | Time | Reference method |
|---|---|---|---|
| 1. Milk | 145ºF (62.8ºC) | 30 minutes | LTLT |
| | 161ºF (71.7ºC) | 15 seconds | STHT |
| | 191ºF (88ºC) | 1 second | UHT |
| | 194ºF (89ºC) | 0.5 second | |
| | 201ºF (94ºC) | 0.1 second | |
| | 204ºF (96ºC) | 0.05 second | |
| | 212ºF (100ºC) | 0.01 second | |
| 2. Milk products of 10% fat or more or added sugar (half/half, cream, chocolate milk) | 150ºF | 30 minutes | |
| | 166ºF | 15 seconds | |
| | 191ºF | 1 second | |
| | 194ºF | 0.5 second | |
| | 201ºF | 0.1 second | |
| | 204ºF | 0.05 second | |
| | 212ºF | 0.01 second | |
| 3. Eggnog and frozen dessert mixes | 155ºF | 30 minutes | |
| | 175ºF | 25 seconds | |
| | 180ºF | 15 seconds | |

pasteurizer units need to be routinely opened and closely evaluated for stress cracks, pin holes, gasketing, cleaning, etc. Holes in regenerator and cooling plates can develop and cause contamination.

IP Address: 129.132.208.100

*Vat pasteurization*

All milk products processed by a vat pasteurizer must meet the basic requirements for pasteurization as defined by the PMO code. Proper pasteurization is critical for quality pasteurized milk production, and the following items must be assured. Recording and indicating thermometers must be present and functioning properly. A headspace heater functioning at −15ºC (5ºF) above minimum pasteurization temperatures is necessary to ensure that any product that enters into the headspace is also properly pasteurized.

Vat pasteurization systems can develop a variety of serious problems such as lack of proper controls, leaking valves, improperly operated headspace heaters, and other serious defects. Controls must be accurate, valves and connections must not contain pockets of cold milk, foam (an excellent insulator) should be minimized in the vat during heating and holding, covers must remain in place during and following heating, etc.

*Post-pasteurization contamination*

Commercial dairy processors and/or individual goat dairy operators should attempt to minimize the amount of handling, exposure to the plant environment, and time/temperature abuse of the product after pasteurization (i.e., holding at

© Woodhead Publishing Limited, 2010

elevated temperatures for extended periods of time). This can be accomplished by keeping the number of processing steps and storage time to the minimum after pasteurization.

The dairy processing manager/operator should review the adequacy of cleaning procedures for all processing and filling equipment, as well as piping. Potential areas of post-pasteurization contamination should be routinely checked and corrected. Environmental contamination of product and product contact surfaces should be minimized at all times. Exposure to these hazards of contamination may be minimized by using additional care and shielding.

It is important to determine that proper sanitizers are being used at the appropriate strength and contact time. Cleaning and sanitizing regimens should be reviewed for proper times, temperatures, pressures, and flow rates. The effectiveness of the cleaning and sanitizing regimen has to be included in this review and assessment.

One of the main contamination sources for pasteurized dairy products can be from the contaminated sweet-water and leaking plates. Sweet-water and glycol of chill-water systems has to be thoroughly checked. A scheduled review program should be initiated to ensure that they are properly protected and do not contain harmful organisms. Any equipment, such as storage tanks, jacketed vessels, etc., that utilize sweet-water or glycol solutions should be routinely monitored periodically for leaks and cracks.

Improper absorbent items, brushes, sponges, wooden tools, cracks and crevices in silo tanks, leaking valves, agitator shafts, shielding, and venting can all be sources of harborage and spread of microorganisms in the plant environment. These areas should be carefully monitored on a scheduled basis. Also, the use of impervious materials (i.e., plastic or metal) is recommended to prevent bacterial growth and post-pasteurization contamination of the processed products.

IP Address: 129.132.208.100

In order to prevent post-pasteurization contamination, any product recovered from defoamer systems should be protected from contamination, maintained at or below 7.1ºC (45ºF) at all times, and be repasteurized. The handling of imperfectly capped or filled containers/packages must be thoroughly checked. Elimination of manual handling/filling/capping of containers/packages should be actively sought, because product contamination can also occur at filling/packaging operations. Mandrels, drip shields, bottom and top breakers, prefilling coding equipment, cutting blades, drain tables, box molders and brine tanks are critical areas where environmental contamination may occur. Overhead shielding, conveyor belts, chain rollers, and lubricants should be constantly monitored to avoid possible contamination of the finished products.

## 12.3 Factors affecting quality of goat milk

### 12.3.1 Factors affecting composition and yield of milk

Composition and yield of goat milk can be influenced by many factors including diet, breed of animal, stage of lactation and environmental temperature, etc. The

© Woodhead Publishing Limited, 2010

**Table 12.8** Factors affecting composition and yield of milk

| | | |
|---|---|---|
| Species | Age and body weight at kidding | Diseases |
| Breed | Feed (diet); plane of nutrition | Gestation and length of dry period |
| Individual animal | Season | |
| Stage of lactation | Environmental temperature and humidity | Parity |
| Colostrum | | Genetic polymorphism |

major factors affecting composition and yield of goat milk are listed in Table 12.8, and further delineated in detail below.

*Diet*

Composition of goat milk is affected by diet which appears to be similar to that of cow milk, although some variations of results have been observed (Juàrez and Ramos, 1986). Merlin *et al.* (1988) reported that type of feed affected the total protein content of milk, whereas fat content was not changed. An increased dietary energy content for high-producing goat breeds during lactation has been shown to augment milk production and reduced the fat content (0.2–0.4%), while increasing the nitrogen content (0.1–0.15%) (Fehr and Le Jaouen, 1976; Juàrez and Ramos, 1986). Dahlborn (1987) reported that water deprivation for 48 hours from milking goats resulted in reduction of milk yield, and higher lactose and protein contents.

The role of dietary crude fiber levels on milk fat depression was observed in goat milk by Calderon *et al.* (1984). Restricting roughage and high level of concentrate diets causes a decrease in the level of dietary fiber. At least 17% crude fiber in the diet of the cow is also required to prevent a depression in milk fat (Schmidt, 1971). In a review on the effects of nutritional manipulation on composition and yield of goat milk, Morand-Fehr and Sauvant (1980) reported that goats fed a high concentrate diet (about 15% of their total requirements from concentrates) underwent nearly a 20% increase in milk yield, with a slight decrease in fat content and increases in lactose and protein contents. Similar results in dairy goats were observed in depression of fat content to those in dairy cows, and there were no differences in either milk protein or total solids composition with the high-concentrate diet. Low-fat diets resulted in a drop in the milk fat content (Le Jaouen, 1972), while adding protected lipids led to an appreciable increase in milk fat content (Morand-Fehr and Flamant, 1983). On the other hand, high protein diets did not change the nitrogen content of the goat or cow milks (Vignon, 1976). Higher protein supplementation to the diet above the normal recommended standards had no effect on milk yield but caused only a slight increase in the non-protein nitrogen content of cow milk (Juàrez and Ramos, 1986).

*Breed*

Breed of goats has a significant effect on yield and composition of milk. The Saanen breed produces a high amount of milk but somewhat low fat levels,

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

whereby it is best known as the Holstein of the goat world (Haenlein and Caccese, 1984). The other extreme case is the Nubian breed, which is equivalent to the Jersey breed of cows. Milk yield of Nubian breed is lower with a higher level of solids, including fat and solids-not-fat (SNF). The Toggenburg, LaMancha, Oberhasli and Alpine breeds produce milk yield and composition in between the Saanen and Nubian (Haenlein and Caccese, 1984).

Ramos and Juàrez (1981) found that variation in the fat content among the different dairy goat breeds was greater than variation in protein content. The fat and total solids contents of the milk of imported Alpine, Saanen and Anglo-Nubian breeds in tropical environments were lower than those of the same breeds raised under temperate climate conditions, which might be attributed to both improper diet and the higher temperatures (Juàrez and Ramos, 1986). Indigenous breeds such as West African Dwarf and Red Sokoto have reportedly much richer solids composition but lower yields compared to those of the Swiss breeds.

*Stage of lactation*

Goat milk yield and composition are also influenced by stage of lactation of the milking goats. Le Jaouen (1987) showed that a relatively high level of milk production in the dairy goats starts at kidding and continues to increase to a peak approximately 3–4 weeks after freshening (Fig. 12.4), whereby a similar trend would occur in dairy cows with a peak at 3–6 weeks (Schmidt, 1971). The high milk yield may be held for a few weeks, then the milk production gradually declines toward the end of lactation (Fig. 12.4).

IP Address: 129.132.208.100

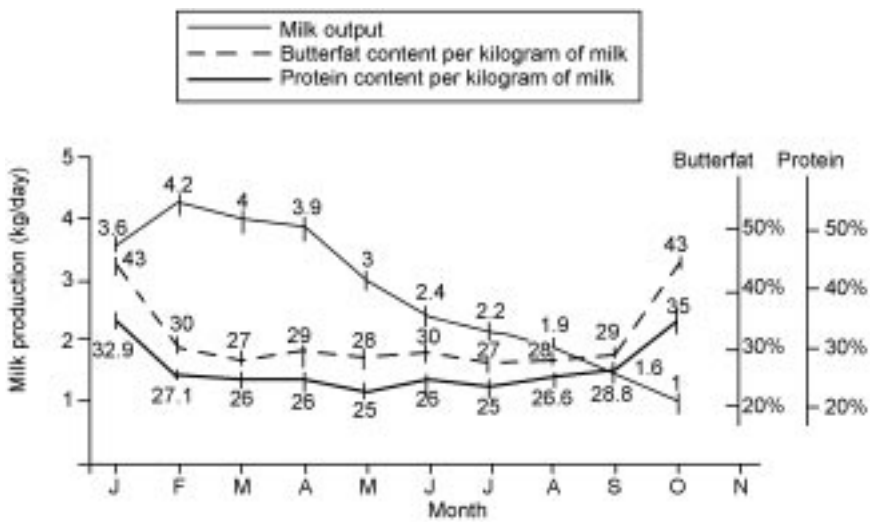



**Fig. 12.4** Changes in milk yield, fat and protein contents during different stages of lactation in goat milk (Le Jaouen, 1987).

© Woodhead Publishing Limited, 2010

At the early stage of lactation, the fat, solids-not-fat, and protein contents of cow milk are high, decrease rapidly, and reach a minimum during the second to third months of lactation, and then increase towards the end of lactation (Schmidt, 1971). Figure 12.4 indicates that there is an inverse relationship between the levels of these components and the yield of milk. In goat milk, fat and protein contents decrease from onset of lactation to the fourth or fifth month and then remain low for a variable length of time, increasing at the end of lactation. During different stages of lactation, lactose content in both goat and cow milk shows some fluctuations, which is different from the trend of fat and protein contents (Renner, 1982; Larson, 1985). The levels of fat and protein are first low, rising in mid-lactation and declining again at the end of lactation in both species' milks.

*Season*

Fat content of milk may vary as much as 2% and SNF as much as 1%, depending on the season. Fat and SNF levels in goat milk vary with season (Fig. 12.4). In temperate climates, late summer milk contains the lowest fat and SNF contents (Parkash and Jenness, 1968; Chandan *et al.*, 1992). Because of the seasonal deviations in levels of fat and protein in milk, a direct reflection of the variations in cheese yield was demonstrated in a Canadian study (Irvine, 1974).

Different kidding seasons result in different milk compositions. Natural breeding usually results in goats freshening at the beginning of spring, although artificial breeding methods are practiced for year-round milking. Chandan *et al.* (1992) reported that the seasonal variations in milk composition are concomitantly involved with lactation stages in overall observed fluctuations of milk constituents. The availability of fresh forages during different seasons also has a significant effect on milk composition and yield.

IP Address: 129.132.208.100

*Environmental temperature*

The effect of environmental temperature on milk yield and composition is dependent upon the species and breed within species of the animal. In dairy cows, Holsteins and the larger breeds are somewhat more tolerant of the lower temperatures, whereas the smaller breeds, especially the Jersey, and to some extent the Brown Swiss, are much more tolerant of the higher temperatures (Schmidt, 1971). Low temperatures do not have a significant effect on the milk yield if extra feed is provided to cover the extra energy required to maintain body temperatures. Milk production is not affected by temperature changes between 4.4 and 21ºC (40–70ºF) when the relative humidity ranges between 60 and 80% (Moody *et al.*, 1967; Schmidt, 1971).

Above the range of thermal neutrality, a considerable decrease in milk production occurs with an increase in environmental temperature. At high temperatures, the feed consumption decreases and the water consumption increases. The feed consumption and milk production approach zero at about 40.6ºC (105ºF) (Schmidt, 1971). When temperatures are below 75ºF, the milk fat, solids-not-fat and total solids percentages increase. At high temperatures, the

© Woodhead Publishing Limited, 2010

chloride content in milk increases while the lactose content decreases. Although dairy goats may be more tolerant to environmental temperatures, few studies have been reported on the effect of environmental temperatures on the yield and composition of goat milk.

*Disease*

Mastitis has a significant effect on both the yield and composition of milk, because it alters the permeability of the udder tissue and impairs the ability of secretory tissue to synthesize milk constituents. Mastitis also destroys the secretory tissue in the udder, which in turn reduces milk production (Schmidt, 1971).

Subclinical and clinical mastitis cause a rise in milk sodium and chloride and a fall in potassium and lactose, with the net result of an increase in electrical conductivity (Fernando *et al.*, 1982). Mastitis also results in an increase in the globulin level, smaller increases in serum albumin and proteose contents, and a decrease in the casein content. Waite and Blackburn (1957) reported that milk with a cell count less than 100,000 cells/ml had no subclinical mastitis and no change in the chemical composition of the milk. As the cell count rose from 100,000 to 500,000 cells/ml, there was a decrease in the SNF and lactose contents of the milk. When the cell count was over 1,000,000 cells/ml, the casein content began to decrease. However, mastitic conditions are not clearly correlated with cell counts in goat milk, whereby the SCC may not represent the changes in composition and yield of goat milk (Park and Humphrey, 1986).

IP Address: 129.132.208.100

*Colostrum*

Colostrum (milk from 1–5 days after parturition) usually contains higher nutrients than normal mature milk, including total solids, protein and ash contents. The most remarkable difference between colostrum and normal milk is protein content, especially immunoglobulin content. The immunoglobulins containing antibody can be absorbed by the newborn during its first day of life. After the first day, due to changes in the absorptive ability of the intestine, the digestive enzymes break down the globulins so that they lose their ability to protect the animal.

Colostrum contains 10 times as much vitamin A as mature milk. Colostrum is also higher in calcium, magnesium, phosphorus, and chlorine, and lower in potassium than normal milk (Schmidt, 1971). Colostrums of cows and goats have a similar secretory pattern of nutrient composition.

*Other factors*

Milk composition and yield can also be influenced by other factors, including age and body weight at kidding, gestation and dry periods, body condition at kidding, plane of nutrition, etc. In terms of plane of nutrition, increasing the energy intake increases the level of milk production toward the goat's inherited potential. Factors that increase milk yield in cows or goats are increased body weight, advancing age, increased plane of nutrition, fall and winter kidding,

© Woodhead Publishing Limited, 2010

moderate or cool environmental temperatures, and good body condition at kidding.

### 12.3.2 Factors affecting quality of goat milk before and after milking

Milk is subjected to rapid deterioration, since it is an excellent culture medium for bacteria and easily changed by the environmental conditions such as light, temperature and oxygen. Hygienic milking environments and milk handling before and after milking are an essential prerequisite for the production of quality goat milk and its products.

*Factors affecting quality of goat milk before and during milking*

As specified in Table 12.9 and previously described, proper management of feeding with a balanced diet, proper handling and cleaning of milking barns and milking parlor, and proper ventilation before and during milking are essential for the production of grade A quality goat milk. In order to produce grade A quality raw milk, milking goats have to be maintained as a healthy and mastitis-free herd. Table 12.10 depicts the five-point mastitis control program for reduction of somatic cell counts (SCC) promoted by the National Mastitis Council, USA. For production of high quality low-SCC milk, it is recommended to carefully follow the five-point mastitis control program shown in Table 12.10.

In further consideration of factors affecting production of quality goat milk prior to and during milking, any chances of getting objectionable odors into the

IP Address: 129.132.208.100

**Table 12.9** Factors affecting composition and quality of milk before and after milking

| |
|---|
| Management and dietary factors: |
| Proper feeding (i.e., balanced diet, amount of feeding) |
| Percent of roughage feeding |
| Proper handling of animals |
| Proper ventilation |
| Proper cleaning of barns and milking parlor |
| Prior and during milking: |
| Feeding and handling (i.e., objectionable odor) prior and during milking |
| Use of recommended detergent, acid and sanitizers |
| Cleanliness of udder and teats, as well as milking workers |
| Use of properly functioning and clean equipment |
| Handling of the milk during and after milking |
| Attentiveness of dairy plant workers |
| Post milking and processing: |
| Cooling |
| Transportation |
| Pasteurization |
| Processing |
| Packaging |
| Processing utensils |
| Post-pasteurization contamination |

© Woodhead Publishing Limited, 2010

**Table 12.10** The five-point mastitis control program for reduction of somatic cell counts promoted by the National Mastitis Council, USA

| | |
|---|---|
| 1. | Use only functionally adequate milking machines, or hand milking in the correct manner. |
| 2. | Dip teats after each milking with an effective, approved product. |
| 3. | Administer promptly a full series of recommended treatments to all clinical cases of mastitis. |
| 4. | Treat udder halves at drying-off of goats with an approved antibiotic preparation for drying-off. |
| 5. | Cull animals with chronic infections when they do not respond to treatments. |

milk should be prevented, and also udder, teats, milking equipment and milking personnel should be in clean sanitary conditions. The workers should use the recommended detergent, acid and sanitizers, and should routinely check the cleanliness and functioning of the milking equipment and other auxiliary tools and equipment.

*Factors affecting quality of goat milk after milking*

The factors associated with the production of quality goat milk after milking as well as at the milk processing premises are also shown in Table 12.9. These factors include, but are not limited to, cooling the raw milk, transportation of the raw milk to the milk processing facility, pasteurization and processing, packaging, handling the processing equipment and utensils, and post-pasteurization contamination.

To keep high quality goat milk after milking, the raw and pasteurized milks have to be carefully monitored to prevent off-flavor development by lipolysis. Three types of lipolysis can occur: (i) induced, (ii) spontaneous, and (iii) microbial lipolysis (Deeth and Fitz-Gerald, 1976). Induced lipolysis can occur by farm factors, processing factors and dairy plant factors, including transportation, agitation and foaming, homogenization, activation by temperature changes, freezing and thawing, mixing, separation, poor refrigeration, etc. Spontaneous lipolysis can occur through two main factors: (i) milk processing factors, such as cooling, mixing, and separation, which disrupt milk fat globule membranes; and (ii) milking animal factors such as stage of lactation, feed, season, breed, mastitis, milk and fat yield, and physiological factors. In microbial lipolysis, many microorganisms contaminate dairy products, produce lipase, and can cause the development of rancid flavor. The psychrotrophic bacteria are most common sources of these lipases. Bacterial lipases are different from milk lipases, are not inactivated by pasteurization, and can attack the intact fat globules in milk (Deeth and Fitz-Gerald, 1976).

Pasteurization temperature and conditions must be strictly enforced for the PMO regulations in order to ensure the production of quality grade A pasteurized goat milk. Any product that has been mishandled, not protected from contamination, or not maintained at a temperature of 7.1ºC or less, should be discarded. External carton contamination with *Listeria* and *Yersinia* has

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

occurred and may lead to product contamination. All milk and milk products that have overflowed, leaked, been spilled, or been improperly handled are discarded. When the handling and/or refrigeration of such milk and milk products are not in compliance with this requirement, they should be discarded. Milk and milk products from damaged, punctured or otherwise contaminated containers or product from out-of-code containers should not be repasteurized for Grade A use.

## 12.4 Developments in processing techniques for goat milk

Due to the smaller scale of milk production than for cow milk and the seasonal milk supply, the goat milk industry cannot compete with its dairy cow counterpart in terms of total volume of production. This species-specific inherent disadvantage of the dairy goat industry necessitates exploration of some alternative and advanced technological solutions for year-round supply of dairy goat products as well as enhancing quality, productivity, storage and distribution of the products.

### 12.4.1 Pasteurization and heat treatment methods for goat milk

Goat milk has a great sensitivity toward heat treatment (Chandan *et al.*, 1968; Lavigne *et al.*, 1989), whereby it is usually unable to withstand UHT treatment (Patton *et al.*, 1980). Several methods, including pH adjustment, addition of a calcium sequestrant, and preheating of milk, have been proposed to improve the heat stability of goat milk and to sustain UHT treatments for goat milk (O'Connor and Fox, 1973; Patton *et al.*, 1980). The problem of stability in high temperature treated goat milk was not resolved due to rapid destabilization and flavor alteration of UHT processed fluid goat milk.

IP Address: 129.132.208.100

On the other hand, in recent years, goat milk has been routinely processed by the UHT method in commercial goat dairies such as in the Wisconsin dairy goat cooperative (Wisconsin, USA). Great variability in heat stability exists between individual milk samples. Heat coagulation times at 140ºC were between 0.5 and 23.4 min (Chandan *et al.*, 1968), while heat coagulation temperatures of individual goat milk samples ranged from 118ºC to more than 140ºC (O'Connor and Fox, 1973).

Lavigne *et al.* (1989) showed that the high-temperature short-time (HTST) pasteurization method was the best processing method for goat milk to preserve various vitamins as well as extend the shelf-life. The HTST, flash and UHT processes were better than LTLT (low-temperature long-time) and autoclave treatment methods in preservation of thiamine, riboflavin and vitamin C in goat milk (Lavigne *et al.*, 1989).

Pasteurization is performed according to the US FDA standards (Table 12.7) or EU standard. In general, manual and batch pasteurization of milk is performed at 145ºF (62.8ºC) for 30 minutes. Georgia Small Ruminant Research

© Woodhead Publishing Limited, 2010

and Extension Center, Fort Valley State University, GA, as well as a couple of recently licensed grade A goat dairies in Georgia, use the batch pasteurization method, and the fluid milk is sold whenever it is available during the milk production season and/or extra milk is available beyond the amount used for cheese processing. Many other states including Texas, Wisconsin, California, New York, Pennsylvania and Vermont use the LTLT method if the processing plant is not equipped with automated pasteurization facilities (Park and Guo, 2006).

### 12.4.2 Heat stability of goat milk in relation to processing techniques

Heat stability of milk is greatly important for processing and production of dairy foods, especially in goat milk product manufacture. Most heat stability studies of goat milk have indicated that it has great sensitivity toward heat treatment (Chandan *et al.*, 1968; Lavigne *et al.*, 1989). Goat milk is considerably less stable to heat than bovine milk, suggesting that the former may need more attention in terms of heat processing technique than the latter.

High ionic calcium content and low micellular solvation in caprine milk may contribute to heat instability (Remeuf, 1992). Because of the great sensitivity of goat milk toward heat treatment, it is usually unable to withstand UHT treatment (Patton *et al.*, 1980). In order to improve heat stability of goat milk as well as to sustain UHT treatments for goat milk, several methods have been proposed, including pH adjustment, addition of a calcium sequestrant, and preheating of milk (O'Connor and Fox, 1973; Patton *et al.*, 1980). However, the problem of stability in high temperature treated goat milk has not been resolved due to rapid destabilization and flavor alteration of UHT processed fluid goat milk (Park and Haenlein, 2007).

IP Address: 129.132.208.100

A pronounced heat stability maximum at about pH 6.9, with low heat stability at both low and high pH, was displayed by the heat coagulation time versus pH profile for caprine milk samples typically heated at 140ºC (Fox and Hynes, 1976; Tziboula, 1997). The pH threshold of goat milk is around 6.9, while that of cow milk is 6.5–6.6. This difference in the pH threshold between goat and cow milks may cause the variability in heat instability of goat milk (Patton *et al.*, 1980). Goat milk shows lower heat stability at its original pH of 6.7 when compared to cow milk. The lower heat stability of goat milk may account for the differences between micellar characteristics or salt equilibria of the two milks. Tziboula (1997) postulated that the heat stability of goat milk is dependent on the casein genotype, where goat milk with high $\alpha_{s1}$-CN has a lower heat stability than those with a low $\alpha_{s1}$-CN content.

### 12.4.3 Ultrafiltration process

A new concept for the manufacture of natural cheese called the MMV process was introduced in 1969 by Maubois, Mocquot and Vassal (Kosikowski, 1977, 1986). This technology is based on selective concentration of skim or whole

© Woodhead Publishing Limited, 2010

milk by ultrafiltration (UF) to produce a very high fat and protein liquid, called retentate.

For goat milk processing, the ultrafiltration technique has been used for the production of the retentate, pre-cheese fraction from the milk. This pre-cheese fraction is subsequently made into cheese. This ultrafiltration process resulted in an 8–15% increase in cheese yield, as whey proteins are retained with the curd and less rennet is required for cheese processing (Loewenstein *et al.*, 1980; Kosikowski, 1986). The UF technique has one advantage for the manufacture of a cream cheese-type product by providing the possibility of holding the pre-cheese material in frozen storage for later use (Loewenstein *et al.*, 1980). The UF procedure has also been used to prepare a spray-dried retentate material which can be reconstituted and made into cheese at a later date (Kosikowski, 1977; Pierre, 1978).

Even with a shortage of fluid milk supply, especially in off-season milking, it is now possible to produce goat cheese using the UF technology. The large chèvre cheese manufacturers in France can now even out the production variations throughout the year by using this ultrafiltration technique. A goat milk cheese processing plant in New Zealand has also converted to UF technology. This UF technology will also be adopted in goat milk processing in the US.

### 12.4.4 Freezing goat milk curds and cheeses

Long-term preservation methods for goat milk products are highly desired for marketing the products to consumers during the off-milking season. One way is production of pre-cheese. For manufacture of cheese, the pre-cheese produced by UF is adjusted with plastic cream to the composition of the natural cheese desired. It is inoculated with starter culture, rennet, color, salt and mold spores when necessary, and then the viscous, liquid pre-cheese is poured into plastic forms (Kosikowski, 1986). In this process, the curd is formed with little or no free whey in a short time and no cheese vats are required. Continuous cheese making became possible for certain cheese types by this MMV concept. The MMV process technique has been successfully applied to rennet cheeses, such as cream, Camembert, St Paulin, feta, mozzarella, and fresh acid French-type cheese (Kosikowski, 1986).

Another approach to combat the seasonal goat milk supply is freezing curd and holding it in frozen storage. Portmann (1969) reported that cheese made from this curd generally was less desirable in flavor than that made from the fresh curd. However, recent investigations revealed that frozen storage of plain soft and Monterey Jack goat milk cheese for up to six months was possible and did not have a significant negative influence on the sensory quality of the cheeses (Park *et al.*, 2002). Although the concentrations of some organic acids in the frozen stored cheeses were changed, the effects on sensory quality of the products were not deleterious at least up to three months of storage (Park and Drake, 2005), indicating that practical application of this technology would be feasible for the extended marketing of goat products to overcome their seasonal milk supply.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

### 12.4.5 Nutritional fortification

Nutritional fortification is another technical approach for improving the production of dairy goat products such as fluid milk, cheese and yogurt. Vitamins such as vitamin A and D are routinely supplemented in cow milk, and the same fortifications can be applied to fluid goat milk.

Goat milk is deficient in iron, as is cow milk. There is little argument that iron added to milk not only shows excellent biological availability, but can prevent iron-deficiency anemia (Demott, 1971). However, contamination of milk by metal ions has been known to cause 'oxidized' odor and flavor, as a result of lipid peroxidation of milk fat (Shipe *et al.*, 1978). To prevent off-flavors, it was recommended that an elevated pasteurization temperature (77ºC for 16 s rather than 71ºC for 16 s) be used when whole milk is enriched with ferric iron salts and that it should be accompanied by deaeration of milk prior to addition of ferrous iron salts (Edmondson *et al.*, 1971). In the preparation of iron-enriched nonfat dry milk, it was preferable to concentrate the skim milk before adding ferric compounds and subsequently drying the concentrate (Kurtz *et al.*, 1973).

Superdispersed ferric pyrophosphate iron (SDFe) showed excellent absorption properties and bioavailability compared with other iron sources (Juneja *et al.*, 2004). Because of its high stability, SDFe has been used for the fortification of milk, soft drinks, yogurt, yogurt drinks, ice cream, soups and dressings. It is a novel concept in iron fortification for dairy and other food product applications (Juneja *et al.*, 2004).

Iron in ferrous sulfate form has been added to whey cheese in order to enhance the nutritional quality of goat cheese (Yastgaard *et al.*, 1968). This iron fortification technique has been successfully carried out for cow Cheddar cheese without having any detrimental effects on the cheese quality (Zhang and Mahoney, 1991). Iron fortification in goat milk cheese would be feasible since it has been successfully accomplished in cow milk counterparts.

IP Address: 129.132.208.100

### 12.4.6 Reduced fat goat milk products

A high fat diet is a risk factor for coronary heart disease, atherosclerosis and even diabetes. Consumers are conscientious about dietary fat consumption, such as from meat and dairy products. Numerous studies have been conducted on reduction of fat in cow dairy products, especially in cow milk cheeses, while this line of research on dairy goat products has been almost non-existent. However, production of fat-reduced dairy goat products would be highly desirable for improving goat milk production, especially for the consumers of the dairy goat products.

Reduced-fat and low-fat dairy products such as cheeses that exhibit the characteristics of traditional full-fat cheeses are needed for consumer markets (Honer, 1993). However, fat reduction is associated with many textural and flavor defects in cheeses. Mistry (2001) showed that the texture of reduced-fat Cheddar cheese is described as rubbery, dry and grainy. In order to overcome

© Woodhead Publishing Limited, 2010

textural and flavor defects, the moisture in the nonfat substance in reduced-fat cheese is increased to a level similar to that in the full-fat cheese.

High levels of moisture in reduced-fat cheeses produce pasty cheese that is difficult to shred (Hassan *et al.*, 2005). Stabilizers such as carrageenan increase the water-binding capacity of reduced-fat Cheddar cheese and increase the gel strength (Ma *et al.*, 1997). Lactic acid bacteria are found naturally in all cheese varieties, and are used in the manufacture of fermented dairy products. Exopolysaccharide (EPS)-producing cultures have been used in yogurt, sour cream, Mozzarella cheese, soft cheese, and whipped toppings to improve rheological properties, prevent syneresis, and replace stabilizers (Hassan *et al.*, 1996; Perry *et al.*, 1998; Broadbent *et al.*, 2003).

EPS-producing cultures have recently been used to produce cow milk reduced-fat Cheddar cheese with textural and melting characteristics similar to those of its full-fat counterpart (Awad *et al.*, 2005). Many fat mimetics are available with applications in reduced-fat and low-fat cheeses or processed cheeses (Drake and Swanson, 1995). Although it would be greatly desired, no study has been reported on the development of reduced- or low-fat goat milk cheeses using EPS-producing cultures, nor on fat mimetics.

### 12.4.7 Goat milk yogurt processing

In the manufacture of yogurt, the milk is first standardized to 1.0–1.7% fat, pasteurized, homogenized, and then cooled to the optimal fermentation temperature (42–45ºC) for inoculation of the appropriate fermentative yogurt starter culture bacteria. A commonly used bacterial combination of starter cultures in the US is *Streptococcus thermophilus* with *Lactobacillus delbrueckii* subsp. *bulgaricus*. These two cultures produce lactic acid at a greater rate when used together than when used alone (Tamime and Robinson, 1999).

IP Address: 129.132.208.100

These yogurt starter bacteria metabolize lactose in the milk, and release lactic acid into the milk as a waste product (Walstra *et al.*, 1999). The starters are heterofermentative bacteria, which ferment lactose, a disaccharide containing glucose and galactose units, through the glycolytic pathway. As the bacteria continue to produce more acid, the initial pH of goat milk drops from 6.7 to 4.5 or less (Zadow *et al.*, 1983). As the pH is lowered, calcium phosphate is released from the micelles, causing an increase in calcium content in the serum portion of the milk. Portions of all the major caseins ($\kappa$, $\beta$, $\alpha_s$) are dissociated from the micelles at pH 5.6. This disruption of the core structure of the casein micelles causes a loss of stability and leads to aggregation (Holt and Horne, 1996).

The casein particles begin to aggregate at pH 5.2, due to decreased repulsive forces, which allow hydrophobic interactions to take place to form structures with empty spaces between them (Park and Guo, 2006). Contraction of casein aggregates takes place between pH 5.2 and 4.8, and these particles are larger than the original micelles in the milk. At pH 4.5 or below, rearrangement and aggregation of casein particles occur, which leads to the formation of a protein matrix consisting of micellar chains and clusters trapping other milk components

© Woodhead Publishing Limited, 2010

inside. After all these steps, a milk gel is formed as yogurt (Tamime and Robinson, 1999).

A variety of methods have been used to improve the texture of yogurt for many centuries. Improving goat milk yogurt would be very important, since it has been known to have an inferior texture compared to its cow milk counterpart. The most commonly employed technique is to increase the amount of total solids in the milk. The general rule is that the higher the level of solids in the milk, the greater the viscosity and textural consistency of the yogurt.

*Enhancing goat milk yogurt texture by ingredient fortification*

A common method to increase total solids content is boiling milk, especially in rural areas for small-scale yogurt manufacture. The milk is boiled until the volume is reduced to two-thirds of its original volume. Boiling concentrates the milk and modifies the properties of the casein, which in turn improves the viscosity of the final product. However, boiling can alter sensory characteristics and cause a loss of heat-labile vitamins (Tamime and Robinson, 1999).

The addition of powder ingredients is another method to increase solid content of the milk which makes a thicker and smoother yogurt. Skim milk powder, whey products, and buttermilk powder are usually used. Goat milk yogurt with good body texture and flavor is most realistically achieved by supplementation of goat milk with cow skim milk powder. Increasing the level of total solids to 15% in goat milk with cow skim milk powder increased the rate of lactic acid production, masked the goaty flavor and decreased syneresis (Agnihotri and Prasad, 1993).

IP Address: 129.132.208.100

Additives such as sodium caseinate or micellar casein are also utilized to improve yogurt texture (Keogh and O'Kennedy, 1998). Because casein forms the gel structure in yogurt, its structure will become stronger if more casein is added (Tamime and Robinson, 1999). Casein is also an effective additive because casein and $\beta$-lactoglobulin interact chemically on heating, which effectively increases the concentration of gel-forming protein in the yogurt matrix and reduces syneresis through increased entrapment of serum within the interstices of the whey protein molecules attached to the surface of the casein (Keogh and O'Kennedy, 1998).

Improvement of yogurt texture by increasing total solids has been achieved by other methods, including concentration by vacuum evaporation, concentration by membrane filtration, and addition of non-milk proteins, such as soy protein, egg white, ground nut protein, etc. (Park and Guo, 2006).

Stabilizers (polysaccharides and common natural or synthetic gums) are also utilized as an additive to enhance and maintain the texture of yogurt (White, 1995). Stabilizers improve the texture of yogurt in two ways: (a) they bind water, and (b) they form a network of linkages between milk constituents and themselves. This is achieved by the presence of a negatively charged group, such as hydroxyl or carboxyl radicals, or by the presence of a salt, possessing the power to sequester calcium ions, which are parts of the gel structure in untreated yogurt (Tamime and Robinson, 1999).

© Woodhead Publishing Limited, 2010

*Improving goat milk yogurt texture by enzymatic crosslinking*

An enzymatic method using microbial transglutaminase (MTGase) has been developed to modify the texture of yogurt. Transglutaminase is an important enzyme for many biological processes in many organisms including fibrin clot stabilization, hair follicles, and crosslinking of erythrocytes (Nio *et al.*, 1985).

One microorganism, *Streptoverticicillium mobaranese*, was shown to produce a high level of transglutaminase-like enzyme, while only barely detectable levels of the enzyme could be produced by other microorganisms (Ando *et al.*, 1989). This transglutaminase (MTGase) has the same crosslinking abilities as animal and plant transglutaminase (Ando *et al.*, 1989). This enzyme has been labeled as microbial transglutaminase (MTGase) (Motoki and Seguro, 1998), which is presently commercially produced by Ajinomoto Inc., IA, USA (Motoki and Kumazawa, 2000). The MTGase has been used in the food industry to crosslink many varieties of protein, including whey, soy, wheat, beef myosin, and casein (Zhu *et al.*, 1995).

MTGase has been elucidated for its physical and chemical characteristics by recent studies (Motoki and Kumazawa, 2000; Motoki and Seguro, 1998). Its molecular weight is 40 kDa, and its iso-electric point is 8.9. The primary structure comprises 331 amino acid residues with a molecular weight of 37,842. MTGase was also shown to contain a single cysteine residue essential for its catalytic activity.

Researchers have also reported that MTGase catalyzes acyl-transfer reactions, which covalently crosslink the lysine and glutamine ends of various protein molecules, forming larger protein complexes from small protein substrates (Zhu *et al.*, 1995; Lauber *et al.*, 2000; Motoki and Kumazawa, 2000; O'Sullivan *et al.*, 2001). A crosslink is often catalyzed between glutamine and lysine, the $\epsilon$-($\gamma$-glutamyl) lysine crosslink (Fink *et al.*, 1980). The protein crosslinkages not only improve the yogurt structure but produce non-protein nitrogen, which could contribute to increased growth of *S. thermophilus* (Park and Guo, 2006).

IP Address: 129.132.208.100

Recently, a texture-improved probiotic goat milk yogurt produced by enzymatic crosslinking has been developed at the University of Vermont (Farnsworth, 2003; Park and Guo, 2006). The consistency of the yogurt was greatly improved by the addition of MTGase. Scanning electron micrographs revealed that the microstructure of the yogurt treated with MTGase became increasingly dense as the MTGase level was increased from 0 to 2 and 4 units per gram protein (Park and Guo, 2006). Enzymatic crosslinking did not have a significant impact on the survival of the probiotic cultures *L. acidophilus*, *L. casei* and *Bifidobacteria* ($P > 0.05$) or on the chemical composition of the yogurt, including total solids, ash, lactose, protein, fat, and mineral content ($P > 0.05$). Enzymatic crosslinking by MTGase has great potential to improve goat milk yogurt consistency (Farnsworth, 2003).

© Woodhead Publishing Limited, 2010

### 12.4.8 Evaporated and powdered goat milk products

Little research has been reported on evaporated and powdered goat milk products, while they are manufactured in the US and New Zealand, and marketed around the world (Park, 2000; Park and Guo, 2006). Evaporation is usually done under reduced pressure, primarily to allow boiling at a lower temperature and thus prevent flavor damage due to heating. The principal components of an evaporation plant are (a) evaporation chambers operating as heat exchangers, (b) equipment for the production and maintenance of a vacuum, (c) separators for the separation of vapor and concentrate, and (d) a condenser for the vapor (Cross and Overby, 1988; Park and Guo, 2006). The general composition of evaporated cow milk is 7.5–9.0% fat, 17.5–22% milk solids nonfat, and 25–31% total solids, while that of its goat milk counterpart is shown in Table 12.11.

In manufacturing powdered milk, two different processing methods are used for dried milk products: roller drying and spray drying. In the roller drying process, milk or milk concentrate is applied in a thin film on the surface of a rotating, steam-heated metal drum (Park and Guo, 2006). During the process of rotation, the milk film dries and is continuously scraped off by a stationary knife located opposite the point of application of the concentrate. In the spray drying process, fluid milk is transformed into a dried particulate by spraying the milk into a hot drying medium. Four process stages of conventional spray drying include (i) atomization of milk into a spray, (ii) spray drying air contact (mixing and flow), (iii) drying of spray (water evaporation), and (iv) separation of dried product from the air (Cross and Overby, 1988).

IP Address: 129.132.208.100

### 12.4.9 Frozen goat milk products

Goat milk ice cream can be manufactured, but little research has been documented (Loewenstein *et al.*, 1980). Three formulations made for three flavors of goat ice cream may be (i) French vanilla mix with 14% fat, 10% MSNF, 18% sweetener (12% sucrose, 6% 36-dextrose equivalent corn syrup solids), 1.4% egg yolk solids, and 0.25% stabilizer–emulsifier; (ii) chocolate mix: 14.6% fat (0.6% cocoa fat), 9% MSNF, 20% sweetener (14% sucrose, 6% 36-DE corn syrup solids), 3% medium fat cocoa, and 0.22% stabilizer–emulsifier; (iii) premium white mix: 15% fat, 10% MSNF, 18% sweetener and 0.25% stabilizer–emulsifier (Park and Guo, 2006).

### 12.4.10 Cosmetic goat milk products

Production and marketing of cosmetic goat milk products are definitely the emerging sectors of the goat milk industry. Cosmetic products made from goat milk have become increasingly popular recently; these include goat milk soap, hand lotion, etc. These products are produced on a commercial scale in the US and other countries such as Switzerland. A list of more than 5000 references appeared from an Internet search on goat milk soap. There has been a

© Woodhead Publishing Limited, 2010

**Table 12.11** Basic nutrient contents (%) of commercial US goat milk products (wet basis)

| Goat milk product | Total solids | | Protein | | Fat | | Carbohydrates | | Ash | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $\bar{X}$ | SD | $\bar{X}$ | SD | $\bar{X}$ | SD | $\bar{X}$ | SD | $\bar{X}$ | SD |
| Fluid milk | | | | | | | | | | |
| Recent study[a] | 11.3 | 0.05 | 2.92 | 0.09 | 3.40 | 0.10 | 4.15 | 0.13 | 0.79 | 0.01 |
| USDA[b] | 13.0 | 0.15 | 3.56 | 0.03 | 4.14 | 0.05 | 4.45 | – | 0.82 | 0.01 |
| Evaporated milk | | | | | | | | | | |
| Recent study[a] | 20.85 | 0.05 | 6.11 | 0.33 | 6.75 | 0.05 | 6.56 | 0.53 | 1.43 | 0.10 |
| USDA[c] | 25.86 | 0.08 | 6.81 | 0.03 | 7.56 | 0.01 | 10.04 | – | 1.55 | 0.02 |
| Powdered milk | | | | | | | | | | |
| Recent study[a] | 94.1 | 0.56 | 27.0 | 0.45 | 28.2 | 1.35 | 32.0 | 0.33 | 6.77 | 0.15 |
| USDA[d] | 97.5 | 0.13 | 26.3 | 0.18 | 26.9 | 0.25 | 38.4 | – | 6.08 | 0.09 |
| Yogurt[e] | | | | | | | | | | |
| Plain | 11.5 | 2.56 | 3.99 | 0.12 | 2.25 | 0.13 | 4.49 | 0.56 | 0.82 | 0.02 |
| Blueberry | 17.7 | 2.34 | 3.37 | 0.13 | 1.18 | 0.17 | 12.6 | 2.72 | 0.86 | 0.09 |
| Cheese[f] | | | | | | | | | | |
| Soft | | | | | | | | | | |
| Plain | 40.2 | 6.81 | 18.9 | 5.26 | 22.5 | 4.37 | – | – | 1.74 | 0.97 |
| Herb | 40.9 | 2.11 | 17.3 | 2.26 | 21.8 | 2.13 | – | – | 1.60 | 0.61 |
| Hard | | | | | | | | | | |
| Cheddar | 58.3 | 1.76 | 30.3 | 0.56 | 26.6 | 1.13 | 1.40 | – | 3.60 | 0.13 |
| Blue | 74.1 | 1.62 | 20.2 | 0.35 | 31.8 | 1.06 | – | – | 3.32 | 0.36 |

[a]Means of eight fluid milk (two brands, four different lots), 12 evaporated milk (two brands, six different lots), and 10 powdered milk (two brands, five different lots) samples, respectively. Data from Park (2000).
[b]Data for fluid goat milk from *USDA Handbook No. 8-1* (Posati and Orr, 1976).
[c]Evaporated canned milk from *USDA Handbook No. 8-1* (Posati and Orr, 1976).
[d]Powdered whole canned milk from *USDA Handbook No. 8-1* (Posati and Orr, 1976).
[e]Park (1994).
[f]Park (1990).
$\bar{X}$ = mean; SD = standard deviation.

IP Address: 129.132.208.100
© Woodhead Publishing Limited, 2010

tremendous increase in the number of home-based goat milk soap businesses in recent years, and these are now estimated to generate multi-billion dollars of annual revenues in the US and around the world (Park and Guo, 2006). Ingredients required for home-made style goat milk soap making include lye, goat milk, borax, oatmeal, pork lard or vegetable oil, etc.

## 12.5 Improving goat milk production

### 12.5.1 Protein polymorphisms in improving goat milk production

Different types of proteins in milk are of great interest for dairy manufacturers, including goat milk producers, for the advancement of processing technology to improve production efficiency and the quality of products. Considering the economic contribution of dairy goat production in France, Spain, Italy, Greece and other countries, it would be important to evaluate the gene frequency of $\alpha_{s1}$-CN alleles in different dairy goat breeds, as well as to determine the effect of the $\alpha_{s1}$-CN genotype as a criterion in selection programs.

There is a direct relationship between protein allelic variants and differences in casein (CN) content, and in physico-chemical properties of milk (Jordana *et al.*, 1996). The firmness of curd formation is very important in the manufacture of hard cheeses, and the amount of $\alpha_{s1}$-casein in milk is directly related to the firmness of curds during protein coagulation as well as cheese yield.

There are six major proteins in goat and sheep milks, and caseins make up nearly 80% of the total protein. Four types of caseins, $\alpha_{s1}$, $\alpha_{s2}$, $\beta$, and $\kappa$, mainly affect cheese yield and quality (Moioli *et al.*, 1998). The genes encoding the four caseins are closely linked, in which the heritable transmitted unit is the haplotype (Grosclaude, 1988). The major whey proteins in goat and cow milk are $\alpha$-lactalbumin and $\beta$-lactoglobulin.

IP Address: 129.132.208.100

Milk protein polymorphisms are caused either by the substitution of amino acids or by the deletion of several of them, and can be detected through electrophoresis of milk and/or analysis of DNA (Moioli *et al.*, 1998). Polymorphism of $\alpha_{s1}$-casein controls the level of $\alpha_{s1}$-casein excretion in milk, and more than 18 allelic genotypes have been identified in goat milk (Tziboula-Clarke, 2003). Each of the alleles $\alpha_{s1}$-CN$^{A}$, $\alpha_{s1}$-CN$^{B}$ and $\alpha_{s1}$-CN$^{C}$ contributes about 3.6 g CN/L milk, whereas $\alpha_{s1}$-CN$^{E}$ contributes only 1.6 g, $\alpha_{s1}$-CN$^{F}$ and $\alpha_{s1}$-CN$^{D}$ 0.5 g, and $\alpha_{s1}$-CN$^{O}$ appears to be a null allele (Jordana *et al.*, 1996). The allele E (medium) is related to an intermediate content, and F and G (weak) are associated with low content of $\alpha_{s1}$-casein (0.5 g/L per allele) (Tziboula-Clarke, 2003). Studies have shown a correlation between the existence of at least seven alleles in the Saanen and Alpine dairy breeds and various amounts of $\alpha_{s1}$-CN in the milk (Table 12.12) (Grosclaude *et al.*, 1987; Mahe and Grosclaude, 1989).

The decreased rate of $\alpha_{s1}$-CN synthesis associated with allele $\alpha_{s1}$-CN$^{F}$ is due to altered RNA splicing, as a consequence of an exonic point deletion (Leroux *et al.*, 1992). The relationship between total casein content (TC) and $\alpha_{s1}$-CN

© Woodhead Publishing Limited, 2010

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

**Table 12.12** Comparison of the allelic frequencies at the CSNS1 locus in Hungarian milking goats with Saanen, Alpine and local Spanish breeds

| | A | B | C+D | E | F | O | References |
|---|---|---|---|---|---|---|---|
| Hungarian milking (103) | 0.09a | 0.29a | 0.08a | 0.08a | 0.46a | 0 | Kusza *et al.* (2007) |
| Alpine (213) | 0.14b | 0.05b | 0.01a | 0.34b | 0.41a | 0.05a | Grosclaude *et al.* (1987) |
| Alpine (80) | – | – | – | 0.35b | 0.59a | 0.06a | Ramunno *et al.* (1991) |
| Saanen (159) | 0.07a | 0.06b | – | 0.41b | 0.43a | 0.03a | Grosclaude *et al.* (1987) |
| Saanen (70) | 0.03a | – | 0.003b | 0.49b | 0.46a | – | Ramunno *et al.* (1991) |
| Murciano-Granadina (109) | 0.08a | 0.23a | – | 0.59b | 0.08b | 0.02a | Jordana *et al.* (1996) |
| Malaguena (373) | 0.09a | 0.09b | – | 0.65b | 0.04b | 0.13a | Jordana *et al.* (1996) |
| Canaria (74) | 0.28b | 0.32a | – | 0.20b | – | 0.20b | Jordana *et al.* (1996) |

Values in the same column with the same letters are not significantly different.
Source: adapted from Kusza *et al.* (2007).

content can be calculated as TC = 19.59 + 0.64$\alpha_{s1}$ (Grosclaude *et al.*, 1987). Quantitative variations of $\alpha_{s1}$-CNs were also reported in Italian goat breeds in relation to the coagulation properties of the goat milk (Ciafarone and Addeo, 1984; Ambrosoli *et al.*, 1988). Brignon *et al.* (1990), studying with the six caprine $\alpha_{s1}$-CN variants, found that variants A and B had the closest homology with the ovine and bovine sequences. The higher amount of $\alpha_{s1}$-CN is likely to be associated with alleles $\alpha_{s1}$-CN$^{A}$, $\alpha_{s1}$-CN$^{B}$, and $\alpha_{s1}$-CN$^{C}$ in goats. These alleles are normally high in caprine species, while the other alleles are defective mutants.

In most European dairy goat breeds such as Alpine, Saanen, Poitevine, Garganica, Maltese, Murciana-Granadina and Malaguena, a polymorphism with seven alleles (A, $B_1$, $B_2$, $B_3$, C, D, E, F, G, O) was found (Table 12.12) (Martin, 1993; Jordana *et al.*, 1996; Moioli *et al.*, 1998; Tziboula-Clarke, 2003). The $\alpha_{s1}$-CNs A, B, C and E differ only by a few amino acid substitutions, while $\alpha_{s1}$-CNs D and F show considerable structural differences, consisting in internal deletions of 11 and 37 residues, respectively, which include the multiple phosphorylation sites (Martin, 1993).

Using PCR-AS and PCR-RFLP methods for 103 goats, Kusza *et al.* (2007) identified the presence of the A, B, C+D, E, F and O alleles of the *CSN1S1* locus (Table 12.12), and A+B+C+E, D, F and O alleles of the *CSN1S2* locus. They found that the strong B allele of *CSN1S1* is more frequent in the local Hungarian milking goats than in the imported Alpine and Saanen goats. They concluded that the relatively high incidence of the O allele of the *CSN1S2* gene was also characteristic for the Hungarian milking goats, which could be used to develop selection strategies for specialized local breeds.

IP Address: 129.132.208.100

### 12.5.2 Effect of milk protein polymorphism on renneting properties

The new knowledge of protein polymorphism could be a challenge and could be rewarding for goat breeding programs, especially since selection for or against $\alpha_{s1}$-casein is now practiced in some countries, due to differences in cheese yield and renneting (Remeuf, 1993; Moioli *et al.*, 1998).

Rennet coagulation of milk proteins is largely dependent on the types of casein present in the milk. Goat milk has a poorer coagulating ability, which is due essentially to its lower casein content and to specific properties of casein micelles such as composition, size and hydration. Storry *et al.* (1983) reported that, on average, the firmness of the gel of goat milk is clearly lower, and the gel from goat milk with an equal casein content is not as firm as from cow milk. Remeuf (1992) also reported that proportions of the four caseins $\alpha_{s1}$, $\alpha_{s2}$, $\beta$, and $\kappa$ are not the same in goat and cow milks, and there are great variations of $\alpha_{s1}$-casein content in individual goat milks due to the occurrence of the genetic polymorphism of this casein, which may have great repercussions on their cheesemaking properties.

When cheesemaking is used for selection, the high frequency of the B$^{IEF}$ (isoelectrofocusing) allele can be exploited in breeding strategies to improve the

© Woodhead Publishing Limited, 2010

protein and casein percentages (Chiatti *et al.*, 2007). Goat milk with the genetic trait of low or no $\alpha_{s1}$-casein, but instead with $\alpha_{s2}$-casein, has less curd yield, longer rennet coagulation time, more heat lability, and weaker curd firmness, which also may explain the benefits in digestibility in the human digestive tract (Ambrosoli *et al.*, 1988; Haenlein, 2004).

The effects of milk protein polymorphism on milk composition and the renneting process have been intensively studied. Remeuf (1993) demonstrated that the seven CN alleles in goat milk are associated with different amounts of $\alpha_{s1}$-CN, where the alleles are correlated with total N, casein and fat content. In addition, $\alpha_{s1}$-CN genetic variants affect micellar size (AA < EE and FF) and micellar mineralization (FF and EE > AA). The three groups of alleles also make for considerable differences in the renneting speed, curd firmness and cheese yield. Best parameters were obtained by homozygous $\alpha_{s1}$-CN AA goats. Allele A gives goat cheese the sweetest flavor, while allele F provides the sharpest taste with respect to the association of alleles with goat cheese taste (Vassal and Delacroix-Buchet, 1994).

### 12.5.3 Keeping and improving goat milk flavor

*General background of goat milk flavor*

The flavor of cow milk and dairy products has been studied extensively, while there has been much less published literature and research on the flavor of goat and other minor species milk. Goat milk has a long history of widespread negative popular perception of 'goaty' flavor, whereby acceptable, attractive milk odor and taste is probably the single most important quality standard of goat milk (Kosikowski, 1986; Haenlein, 1992; Park and Guo, 2006).

IP Address: 129.132.208.100

Goaty odor can be prevented or does not need to occur, because well-produced and well-handled goat milk is not distinguishable in taste and odor from cow milk (Campbell and Marshall, 1975; Mowlem, 1988; Haenlein, 1992). Although goat milk has a higher content of strong-smelling caproic, caprylic and capric acids and probably 4-ethyloctanoic acid in its milk fat, they are enclosed within the fat globule membrane when good milking practices are performed (Park, 2001).

However, the membrane is more fragile in goat milk fat than in cow milk fat, and is easily broken during improper handling, insufficient cooling and repeated rewarming, when enzymes are liberated and cause lipolysis, releasing free fatty acids to produce odors (Loewenstein *et al.*, 1984; Haenlein, 1992; Park, 2001).

*Prevention of sources of off-flavor*

The goat has been the most maligned domesticated animal and still is in many parts of the world (Rubino and Claps, 1995), partly because of its sometimes offensive odor, especially from the buck, whose odor floats strongly around the premises and can affect the flavor of the doe's milk (Haenlein, 2006).

In order to prevent off-flavors and improve the quality and flavor of goat milk for consumers, sources of off-flavors have to be thoroughly identified and

© Woodhead Publishing Limited, 2010

eliminated, and the best possible preventive processing tactics and procedures have to be implemented from the farm level through the milking parlor, processing plant, distribution and storage to the marketing premises. Many sources identified as responsible for off-flavors in cow milk can also be applied to goat milk. The milk of minor species tends to have a more robust flavor, often characterized by a waxy/animal aroma and flavor. Dairy product flavor is caused by an array of chemical reactions including protein reactions, such as Maillard browning, proteolytic, and lipolytic reactions (Carunchia Whetstine and Drake, 2006).

The many sources that can cause off-flavors in domesticated ruminant milks include feeds (molasses, citrus pulp), forages (rape, rapeseed meal), weeds (onions, garlic), environmental odors (oil, gasoline, moldy rags, lime, cement, active bucks), physiological (early lactation colostrums, late lactation high salt contents, estrus, mastitis, sickness), improper hand-milking procedures, improper equipment and handling (oxidation due to risers in milking pipelines, vacuum slips and searches, sun exposure, filthy clogged strainers and pipeline connections, unclean milking equipment, wash water not hot enough), slow air-cooling instead of water-cooling or refrigeration, variable temperature storage, addition of warm milk to cold storage milk, transport in unrefrigerated containers, etc. (Le Jaouen, 1972, 1987; Loewenstein *et al.*, 1984; Peters, 1990; Haenlein, 1992; Park, 2001; Park and Guo, 2006).

Goat milk must be cooled as officially prescribed down to the holding temperature range of 2.2 to 5.5ºC (36 to 42ºF) within a short time after milking, and that temperature should be maintained until processing as well as during transport to a dairy plant (Colorado Department of Health, 1980; Haenlein, 1992; Park and Guo, 2006).

IP Address: 129.132.208.100

### 12.5.4 Somatic cell principles in improving goat milk production

Somatic cell count (SCC) is widely used for evaluating milk quality. An increased SCC results either from an inflammatory process due to the presence of an intramammary infection or, under non-pathological conditions, from physiological processes such as estrus or advanced stage of lactation (Raynal-Ljutovac *et al.*, 2007). An increase in SCC causes a decrease in milk yield and affects milk composition, which leads to reduced cheesemaking potential (Barbano *et al.*, 1991).

In general, the relationship between bacterial counts and SCC in goat milk has not been consistent (Park and Humphrey, 1986). In some cases, bulk tank total bacterial count showed a statistically significant correlation with bulk milk SCC (Gonzalo *et al.*, 2006). This indicates that reduction in SCC by genetic selection and by a tightly controlled sanitary program would reduce milk SCC and improve goat milk production.

The shelf-life of pasteurized milk was lower for high SCC milk. Sensory defects appeared after 14 days at 4ºC for raw milk containing more than 500,000 cells/ml (Rogers and Mitchell, 1994), and bitterness and rancidity after storage for 21 days at 4ºC (Ma *et al.*, 1997). Quality depreciated even more when

© Woodhead Publishing Limited, 2010

psychrotrophic and total microflora developed to an excessive extent in raw milks. On the other hand, no effect on high SCC milk was found on solubility and flavor of milk powders compared to low SCC milk.

### 12.5.5 Increasing CLA and other nutrient contents in improving goat milk

Conjugated linoleic acid (CLA) has gained much attention in recent years because of its several beneficial effects on health, including anticarcinogenic activity (Parodi, 1994; Belury, 1995; Lawless *et al.*, 1998), antiatherogenic activity (Lee *et al.*, 1994; Lawless *et al.*, 1998), the ability to reduce the catabolic effects of immune stimulation (Cook *et al.*, 1993; Lawless *et al.*, 1998), the ability to enhance growth promotion (Chin *et al.*, 1994; Lawless *et al.*, 1998), and the ability to reduce body fat (Pariza *et al.*, 1996; Lawless *et al.*, 1998). CLA is a mixture of positional and geometric isomers of linoleic acid (C18:2) that contain conjugated unsaturated double bonds (Dhiman *et al.*, 1999). The most biologically active isomer of CLA is *cis*-9, *trans*-11-octadecadienoic acid, which accounts for more than 82% of the total CLA isomers in dairy products (Chin *et al.*, 1992; Dhiman *et al.*, 1999).

Research has shown that dietary manipulation can increase the CLA content of goat milk. Feeding canola oil at 2% and 4% of grain intake to Alpine does increased CLA in milk by 88% and 210%, respectively, compared to the non-treated control group (Mir *et al.*, 1999). Dhiman *et al.* (1999) reported that cows fed only on pasture produced milk fat with a higher CLA content than did cows receiving less feed from pasture. This suggests that dairy goats also would produce higher CLA content in goat milk if they were given more pasture feeding conditions.

IP Address: 129.132.208.100

CLA contents in cow milk were substantially increased when animals were fed full-fat rapeseed supplements compared to unsupplemented controls (Lawless *et al.*, 1998). Adding oil rich in unsaturated acids (C18:2–C18:3), which undergo saturation in the rumen, increases the C18:0 and C18:1 acid content (Fehr and Le Jaouen, 1976). Feeding encapsulated lipids in formaldehyde-treated casein led to a marked increase in the proportion of C18:2 and C18:3 acids in the milk (Juàrez and Ramos, 1986), whereby an increase in CLA is possible although not tested. Milk CLA concentration in different ruminant species varied with the season, mainly due to variations in feeding factors (Chilliard and Ferlay, 2004). The greatest seasonal differences were measured in ewe milk, 1.28% in summer and 0.54% at the end of the winter period (Jahreis *et al.*, 1999).

Value-added foods could be produced by increasing CLA content and changing the fatty acid profile in milk by dietary manipulation. Even though the CLA content in dairy products is affected by many factors, animal feeding strategies and specifically diets with seed/oil supplements rich in PUFA have positive effects on CLA content of milks from three species (Stanton *et al.*, 2003; Khanal and Olson, 2004; Chilliard and Ferlay, 2004). Effects of these supplements on milk FA composition in dairy goats (Chilliard *et al.*, 2005) and ewes (Luna *et al.* 2005) have been shown in many studies.

© Woodhead Publishing Limited, 2010

The fatty acid composition of ewe milk, especially in CLA and its precursors, has been studied through feeding trials of fresh forages and different quality of Mediterranean pastures influenced by season (Addis et al, 2005; Cabiddu *et al.*, 2005; Nudda *et al.*, 2005). In addition to enhancing CLA content, the dietary changes incorporating vegetable seeds and oils also result in milk fat containing a lower proportion of saturated FA and greater amounts of monounsaturated FA (including vaccenic acid) and PUFA. Free oils are generally more effective than whole oilseeds, and treated oilseeds have an intermediate effect (Chilliard *et al.*, 2002). Milk yield and fat and protein content response to lipid supplements is different among cows, goats and sheep.

Fish oil is more effective than plant oils in cows for enhancing milk fat CLA content (Chouinard, 2001), and these responses can be further increased when fish oil is fed in combination with supplements rich in C18:2*n*-6 (Abu-Ghazaleh *et al.*, 2003; Jones *et al.*, 2005). Kitessa *et al.* (2001) observed that when goats were fed fish oil, there was a slight increase in $\omega$-3 fatty acid content in goat milk fat, suggesting that dietary supplementations of these oils should be low and protected against ruminal biohydrogenation to avoid a decrease in milk yield, fat and protein content. On the other hand, research data on enhancing CLA content in ewe or goat milk by dietary fish oil supplementation have been very scarce (Mozzon *et al.*, 2002).

## 12.6 Sources of further information and advice

IP Address: 129.132.208.100

Readers are advised to consult with additional references of published books and Web-based reference materials in order to obtain further detailed scientific information on improving goat milk production from, but not limited to, the following sources.

### 12.6.1 Published books

- *Handbook of Milk of Non-bovine Mammals*. 2006. Y.W. Park and G.F.W. Haenlein, eds. Blackwell, Oxford, and Ames, IA.
- *Goat Production*. 1981. C. Gall, ed. Academic Press, London and New York.
- *Goat and Sheep Milk*. 2007. G.F.W. Haenlein, Y.W. Park, K. Raynal-Ljutovac and A. Pirisi, eds. Special Issue, *Small Ruminant Research J.* 68(1–2): 1–232. Elsevier, Amsterdam.
- *Extension Goat Handbook*. 1984. G.F.W. Haenlein and D.L. Ace, eds. USDA Publications, Washington, DC.
- *Encyclopaedia of Dairy Science*. 2002. H. Roginski, J. Fuquay and P. Fox, eds. Elsevier, Amsterdam.
- *The Fabrication of Farmstead Goat Cheese*. 1987. J.-C. Le Jaouen, ed. *Cheesemakers' Journal*, Ashfield, MA.
- *The Small Dairy Resource Book*. 2000. V.H. Dunaway. Sustainable Agriculture Network (SAN), Beltsville, MD.

© Woodhead Publishing Limited, 2010

### 12.6.2 Website reference sources

- Dairygoats.com: www.dairygoats.com
- Sustainable Agriculture Research and Education, SARE/USDA: www.sare.org
- National Agricultural Library, USDA: www.usda.gov
- American Dairy Goat Association: www.adga.org
- A practical guide to small-scale goatkeeping: www.bookfinder.com
- Center for Dairy Research: www.cdr.wisc.edu/
- Maryland Small Ruminant Page: www.sheepandgoat.com
- Technology/Appropriate Technology for Rural Areas/Sustainable Dairy Goat Production: www.attra.ncat.org
- Virtual Library for Dairy Production: www.ansi.okstate.edu/LIBRARY/dairy/
- Small Ruminant and Extension Center, FVSU: www.ag.fvsu.edu
- NCSU Goat Links: http://lenoir.ces.state.nc.us/staff/jnix/Ag/Goat/

## 12.7 References and further reading

ABU-GHAZALEH, A.A., SCHINGOETHE, D.J., HIPPEN, A.R. and WHITLOCK, L.A. 2003. Milk conjugated linoleic acid response to fish oil supplements of diets differing in fatty acid profiles. *J. Dairy Sci.* 86: 944–953.

ADDIS, M., CABIDDU, A., PINNA, G., DECANDIA, M., PIREDDA, G., PIRISI, A. and MOLLE, G. 2005. Milk and cheese fatty acid composition in sheep fed Mediterranean forages with reference to conjugated linoleic acid *cis*-9, *trans*-11. *J. Dairy Sci.* 88: 3443–3454.

AGNIHOTRI, M.K. and PRASAD, V.S.S. 1993. Biochemistry and processing of goat milk and milk products. *Small Rumin. Res.* 12: 151–170.

AMBROSOLI, R., STASIO, L.D. and MAZZOCCO, P. 1988. Content of $\alpha_{s1}$-casein and coagulation properties in goat milk. *J. Dairy Sci.* 71: 24–28.

ANDO, H., ADACHI, M., UMEDA, K., MATSUURA, A., NONAKA, M., UCHIO, R., TANAKA, H. and MOTOKI, M. 1989. Purification and characteristics of a novel transglutaminase derived from microorganisms. *Agric. Biol. Chem.* 53: 2613–2617.

AWAD, S., HASSAN, A.N. and MUTHUKUMARAPPAN, K. 2005. Application of exopolysaccharide-producing cultures in reduced fat Cheddar cheese: Texture and melting properties. *J. Dairy Sci.* 88: 4204–4213.

BARBANO, D.M., RASMUSSEN, R.R. and LYNCH, J.M. 1991. Influence of milk somatic cell count and milk age on cheese yield. *J. Dairy Sci.* 74: 369–388.

BELURY, N.A. 1995. Conjugated dienoic linoleate: a polyunsaturated fatty acid with unique chemoprotective properties. *Nutr. Rev.* 53: 83–89.

BRIGNON, G., MAHE, M.F., RIBADEAU-DUMAS, B. and GROSCLAUDE, F. 1990. Two of the three genetic variants of goat $\alpha_{s1}$-casein which are synthesized at a reduced level have an internal deletion possibly due to altered RNA splicing. *Eur. J. Biochem.* 193: 237–241.

BROADBENT, J.R., MCMAHON, D.J., WELKER, D.L., OBERG, C.J. and MOINEAU, W. 2003. Biochemistry, genetics, and applications of exopolysaccharide production *Streptococcus thermophilus*: A review. *J. Dairy Sci.* 86: 407–423.

CABIDDU, A., DECANDIA, M., ADDISS, M., PEREDDA, G., PIRISI, A. and MOLLE, G. 2005. Managing Mediterranean pastures in order to enhance the level of beneficial fatty acids in sheep milk. *Small Rumin. Res.* 59: 169–180.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

CALDERON, I., DE PETERS, E.J., SMITH, N.E. and FRANKE, A.A. 1984. Composition of goat milk. Changes within milking and effects of a high concentrate diet. *J. Dairy Sci.* 67: 1905–1911.

CAMPBELL, J.R. and MARSHALL, R.T. 1975. *The Science of Providing Milk for Man*. McGraw-Hill, New York.

CARUNCHIA WHETSTINE, M.E. and DRAKE, M.A. 2006. Flavor characteristics of goat milk and other minor species milk products. In: *Handbook of Milk of Non-bovine Mammals*. Y.W. Park and G.F.W. Haenlein, eds. Blackwell, Oxford and Ames, IA, pp. 107–120.

CHANDAN, R.C., PARRY, R.M. and SHAHANI, K.M. 1968. Lysozyme, lipase and ribonuclease in milk of various species. *J. Dairy Sci.* 51: 606.

CHANDAN, R.C., ATTAIE, R. and SHAHANI, K.M. 1992. Nutritional aspects of goat milk and its products. *Proc. V Intl Conf. Goats*, New Delhi, Vol. II, Part II: 399.

CHIATTI, F., CHESSA, S., BOLLA, P., CIGALINO, G., CAROLI, A. and PAGNACCO, G. 2007. Effect of $\kappa$-casein polymorphism on milk composition in the Orobica goat. *J Dairy Sci.* 90: 1962–1966.

CHILLIARD, Y. and FERLAY, A. 2004. Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. *Reprod. Nutr. Dev.* 44: 467–492.

CHILLIARD, Y., FERLAY, A., LOOR, J., ROUEL, J. and MARTIN, B. 2002. Trans and conjugated fatty acids in milk from cows and goats consuming pasture or receiving vegetable oils and seeds. *Ital. J. Anim. Sci.* 1: 243–254.

CHILLIARD, Y., ROUEL, J., FERLAY, A., BERNARD, L., GABORIT, P., RAYNAL-LJUTOVAC, K. and LAURET, A. 2005. Effects of type of forage and lipid supplementation on goat milk fatty acids and sensorial properties of cheeses. In: *International Dairy Federation, special issue, 'The future of the sheep and goat dairy sectors'*, 0501, pp. 297–311.

CHIN, S.F., LIU, W., STORKSON, J.M., HA, Y.L. and PARIZA, M.W. 1992. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *J. Food Compos. Anal.* 5: 185–197.

CHIN, S.F., LIU, W., STORKSON, J.M., ALBRIGHT, K.J., COOK, M.E. and PARIZA, M.W. 1994. Conjugated linoleic acid is a growth-factor for rats as shown by enhanced weight-gain and improved feed efficiency. *J. Nutr.* 124: 2344–2349.

CHOUINARD, P.Y., CORNEAU, L., BUTLER, W.R., CHILLIARD, Y., DRACKLEY, J.K. and BAUMAN, D.E. 2001. Effect of dietary lipid source on conjugated linoleic acid concentrations in milk fat. *J. Dairy Sci.* 84: 680–690.

CIAFARONE, N. and ADDEO, F. 1984. Casein composition and goat milk properties. II. *Vergaro* 11: 17–24.

COLORADO DEPARTMENT OF HEALTH. 1980. *Colorado Grade A Pasteurized Fluid Milk and Milk Products Regulations*, Denver, CO.

COOK, M.E., MILLER, C.C., PARK, Y. and PARIZA, M.W. 1993. Immune modulation by altered nutrient metabilism: nutritional control of immune-induced growth depression. *Poult. Sci.* 72: 1301–1305.

CROSS, H.R. and OVERBY, A.J. 1988. In: *Meat Science, Milk Science and Technology*. H.R. Cross and A.J. Overby, eds. Elsevier, Amsterdam, pp. 349–372.

DAHLBORN, K. 1987. Effect of temporary food or water deprivation on milk secretion and milk composition in the goat. *J. Dairy Res.* 54: 153–163.

DEETH, H.C. and FITZ-GERALD, C.H. 1976. Lipolysis in dairy products: a review. *Aust. J. Dairy Technol.* 31: 53–64.

DEMOTT, B.J. 1971. Effects on flavor of fortifying milk with iron and absorption of the iron

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

from intestinal tract of rats. *J. Dairy Sci.* 54: 1609–1614.

DHIMAN, T.R., HELMINK, E.D., MCMAHON, D.J., FIFE, R.L. and PARIZA, M.W. 1999. Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds. *J. Dairy Sci.* 82: 412–419.

DRAKE, M.A. and SWANSON, B.G. 1995. Reduced- and low-fat cheese technology. *Trends in Food Sci.* 60: 366–369.

EDMONDSON, L.F., DOUGLAS, F.W. and AVANTS, J.K. 1971. Enrichment of pasteurized whole milk with iron. *J. Dairy Sci.* 54: 1422.

FAO. 1997. *Production Yearbook*. Food and Agriculture Organization, United Nations, Rome, 51: 218–222.

FARNSWORTH, J.P. 2003. The effects of enzymatic crosslinking by microbial transglutaminase on the chemical, physical, and microbiological properties of goat milk yogurt. MS thesis, University of Vermont.

FEHR, P.M. and LE JAOUEN, J.C. 1976. Effects of dietary factors on milk composition and characteristics of goats' milk cheese. *Rev. Laitière Franç.* no. 338: 39–55.

FERNANDO, R.S., RINDSIG, R.B. and SPAHR, S.L. 1982. Electrical conductivity of milk for detection of mastitis. *J. Dairy Sci.* 65: 659–664.

FINK, M.L., CHUNG, S.I. and FOLK, J.E. 1980. $\gamma$-Glutamine cyclotransferase: specificity toward $\epsilon$-(L-$\gamma$-glutamyl)-L-lysine and related compounds. *Proc. Natl Acad. Sci.* 77: 4564–4568.

FOX, P.F. and HYNES, M.C. 1976. Heat stability characteristics of ovine, caprine and equine milks. *J. Dairy Res.* 43: 433–442.

GONZALO, C., CARRIEDO, J.A., BENEITEZ, E., JUAREZ, M.T., DE LA FUENTE, L.F. and SAN PRIMITIVO, F. 2006. Short communication: Bulk tank total bacterial count in dairy sheep: factors of variation and relationship with somatic cell count. *J. Dairy Sci.* 89: 549–552.

GROSCLAUDE, F. 1988. Le polymorphisme génétique des principales lactoproteines bovines. [Genetic polymorphism of main milk protein in cattle] *INRA Prod. Anim.* 1: 5–17.

GROSCLAUDE, F., MAHE, M.F., BRIGNON, G., DI STASIO, L. and JEUNET, R. 1987. A Mendelian polymorphism underlying quantitative variation of goat $\alpha_{s1}$-casein. *Genet. Sel. Evol.* 19: 399–412.

GUTHRIE, R.K. 1983. In: *Food Sanitation*, 2nd edn. AVI Publishing, Westport, CT, p. 157.

HASSAN, A.N., FRANK, J.F., SCHMIDT, K.A. and SHALABI, S.I. 1996. Textural properties of yogurt made with encapsulated nonropy lactic cultures. *J. Dairy Sci.* 79: 2098–2103.

HASSAN, A.N., AWAD, S. and MUTHUKUMARAPPAN, K. 2005. Effects of exopolysaccharide-producing cultures on the viscoelastic properties of reduced-fat Cheddar cheese. *J. Dairy Sci.* 88: 4221–4227.

HAENLEIN, G.F.W. 1986. Dimensions of the goat milk industry in the USA. In: Proceedings, Production and Utilization of Ewe's and Goat's Milk Seminar, 23–25 September 1985, Athens, Greece, *Int. Dairy Federation Bull.* 202: 215–217.

HAENLEIN, G.F.W. 1992. Producing quality goat milk. *Nat. Symp. Dairy Goat Prod. Marketing*, Oklahoma City, OK, 12–15 August 1992, pp. 112–127.

HAENLEIN, G.F.W. 1996. Status and prospect of the dairy goat industry in the United States. *J. Anim. Sci.* 74: 1173–1181.

HAENLEIN, G.F.W. 1997. Alternatives in dairy goat product market. *Int. J. Anim. Sci.* 12: 149–153.

HAENLEIN, G.F.W. 2004. Goat milk in human nutrition. *Small Rumin. Res.* 51: 155–163.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

HAENLEIN, G.F.W. 2006. Production of goat milk. In: *Handbook of Milk of Non-Bovine Mammals*. Y.W. Park and G.F.W. Haenlein, eds. Blackwell, Oxford and Ames, IA, pp. 11–33.

HAENLEIN, G.F.W. and CACCESE, R. 1984. Goat milk versus cow milk. In: *Extension Goat Handbook*. G.F.W. Haenlein and D.L. Ace, eds. USDA Publications, Washington, DC, E-1, p. 1.

HOLT, C. and HORNE, D.S. 1996. The hairy casein micelle: evolution of the concept and its implications for dairy technology. *Neth. Milk Dairy J.* 50: 85–111.

HONER, C. 1993. Second thoughts. *Dairy Field*, January.

IDFA. 1998. *IDFA's Dairy HACCP System*. International Dairy Foods Association, pp. 1–44.

IRVINE, D.M. 1974. The composition of milk as it affects the yield of cheese. *Proc. 11th Annual Marshall Invitational Cheese Seminar*, Marshall Div., Miles Lab., Madison, WI.

JAHREIS, G., FRITSCHE, J. and KRAFT, J. 1999. Species dependent, seasonal, and dietary variation of conjugated linoleic acid in milk. In: *Advances in Conjugated Linoleic Acid, Volume 1*, M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza and G.J. Nelson, eds. American Oil Chemists Society, Champaign, IL, pp. 215–225.

JONES, E.L., SHINGFIELD, K.J., KOHEN, C., JONES, A.K., LUPOLI, B., GRANDISON, A.S., BEEVER, D.E., WILLIAMS, C.M., CALDER, P.C. and YAQOOB, P. 2005. Chemical, physical, and sensory properties of dairy products enriched with conjugated linoleic acid. *J. Dairy Sci*. 88: 2923–2937.

JORDANA, J., AMILLS, M., DIAZ, E., ANGULO, C., SERRADILLA, J.M. and SANCHE, A. 1996. Gene frequencies of caprine $\alpha_{s1}$-casein polymorphism in Spanish goat breeds. *Small Rumin. Res.* 20: 215–221.

JUÀREZ, M. and RAMOS, M. 1986. Physico-chemical characteristics of goat milk as distinct from those of cow milk. *Int. Dairy Bull.* no. 202: 54.

JUNEJA, L.R., SAKAGUCHI, N., YAMAGUCHI, R. and NANBU, H. 2004. Iron fortification of dairy products: A novel approach. In: *Handbook of Functional Dairy Products*. C. Shortt and J. O'Brien, eds. CRC Press, Boca Raton, FL, pp. 199–215.

KAPTURE, J. 1980. Somatic counts don't tell whole mastitis story with goat milk. *Dairy Goat Guide* (Dec.) 3: 9.

KAPTURE, J. 1982. An overview of problems in marketing dairy goat products in the U.S.A. In *Proc. Int. Conf. Goats Prod. Dis.*, Tuscon, AZ, 10–15 January 1982, p. 63.

KEOGH, M.K. and O'KENNEDY, B.T. 1998. Rheology of stirred yogurt as affected by added milk fat, protein and hydrocolloids. *J. Food Sci.* 63: 108–112.

KHANAL, R.C. and OLSON, K.C. 2004. Factors affecting conjugated linoleic acid (CLA) content in milk, meat, and egg: a review. *Pakistan J. Nutr.* 3: 82–98.

KITESSA, S.M., GULATI, S.K., ASHES, J.R., FLECK, E., SCOTT, T.W. and NICHOLS, P.D. 2001. Utilisation of fish oil in ruminants – II. Transfer of fish oil fatty acids into goats' milk. *Anim. Feed Sci. Tech.* 89: 201–208.

KOSIKOWSKI, F.V. 1977. *Cheese and Fermented Milk Foods*, 2nd edn. Edwards Brothers, Ann Arbor, MI, pp. 90–108.

KOSIKOWSKI, F.V. 1986. Requirements for the acceptance and marketing of goat milk cheese. *Dairy Goat J.* 64: 462.

KURTZ, F.E., TAMSMA, A. and PALLANSCH, M.J. 1973. Effect of fortification with iron on susceptibility of skim milk and nonfat dry milk to oxidation. *J. Dairy Sci.* 56: 1139–1143.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

KUSZA, S., VERESS, G., KUKOVICS, S., JAVOR, A., SANCHEZ, A., ANGIOLILLO, A. and BOSZE, Z. 2007. Genetic polymorphism of $\alpha_{s1}$- and $\alpha_{s2}$-caseins in Hungarian milk goats. *Small Rumin. Res.* 68: 329–332.

LARSON, B.L., ED. 1985. *Lactation*. Iowa State University Press, Ames, IA.

LAUBER, S., HENLE, T. and KLOSTERMEYER, H. 2000. Relationship between the crosslinking of caseins by transglutaminase and the gel strength of yogurt. *Eur. Food Res. Technol.* 210: 305–309.

LAVIGNE, C., ZEE, J.A., SIMARD, R.E. and BELIVEAU, B. 1989. Effect of processing and storage conditions on the fate of vitamins $B_1$, $B_2$, and C and on the shelf-life of goat's milk. *J. Food Sci.* 54: 30–34.

LAWLESS, F., MURPHY, J.J., HARRINGTON, D., DEVERY, R. and STANTON, C. 1998. Elevation of conjugated *cis*-9, *trans*-11-octadecadienoic acid in bovine milk because of dietary supplementation. *J. Dairy Sci.* 81: 3259–3267.

LE JAOUEN, J.C. 1972. Characteristic and composition of goat milk from zootechnical point of view and as regards its utilization. *II Seminario Nacional de Ovinos y Caprinos*, 30: 2 Dic. Maracaibo, Venezuela.

LE JAOUEN, J.C. 1987. The making of farmstead goat cheeses. *Cheesemaker's Journal*, PO Box 85, Ashfield, MA.

LEE, K.N., KRITCHEVSKY, D. and PARIZA, M.W. 1994. Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* 108: 19–25.

LEROUX, D., MAZURE, N. and MARTIN, P. 1992. Mutations away from splice site recognition sequences might *cis*-modulate alternative splicing of goat $\alpha_{s1}$-casein transcripts. Structural organization of the relevant gene. *J. Biol. Chem.* 267: 6147–6157.

LOENNERDAL, B., KEEN, C.L. and HURLEY, L.S. 1981. Iron, copper, zinc and manganese in milk. *Annu. Rev. Nutr.* 1: 149–152.

LOEWENSTEIN, M., SPECK, S.J., BARNHART, H.M. and FRANK, J.H. 1980. Research on goat milk products: a review. *J. Dairy Sci.* 63: 1631–1648.

LOEWENSTEIN, M., FRANK, J.F., BARNHART, H.M. and SPECK, S.J. 1984. Cultured products made from goat milk. In: *Extension Goat Handbook*. G.F.W. Haenlein and D.L. Ace, eds. USDA Publications, Washington, DC, E-5, pp. 1–5. Also Producing quality goat milk, *ibid.*, E-5, p. 5.

LUNA, P., FONTECHA, J., JUÀREZ, M. and DE LA FUENTE, M.A. 2005. Changes in the milk and cheese fat composition of ewes fed commercial supplements containing linseed with special reference to the CLA content and isomer composition. *Lipids* 40: 445–453.

MA, L., DRAKE, M.A., BARBOSA-CANOVAS, G.V. and SWANSON, B.G. 1997. Rheology of full-fat and low-fat Cheddar cheese as related to type of fat mimetic. *J. Food Sci.* 62: 748–752.

MAHE, M.H. and GROSCLAUDE, F. 1989. $\alpha_{s1}$-$Cn^D$, another allele associated with a decreased synthesis rate at the caprine $\alpha_{s1}$-casein locus. *Genet. Sel. Evol.* 21: 127–129.

MARTIN, P. 1993. Polymorphisme génétique des lactoproteines caprines. *Lait* 73: 511–532.

MELLADO, M., OLIVAS, R. and RUIZ, F. 2000. Effect of buck stimulus on mature and pre-pubertal norgestomet-treated goats. *Small Rumin. Res.* 36: 269–274.

MERLIN, U., ROSENTHAL, I. and MALTZ, E. 1988. The composition of goat milk as affected by nutritional parameters. *Milchwissenschaft* 43(6): 363–365.

MIR, Z., GOONEWARDENE, L.A., OKINE, E., JAEGAR, S. and SCHEER, H.D. 1999. Effect of feeding canola oil on constituents, conjugated linoleic acid (CLA) and long chain fatty acids in goats milk. *Small Rumin. Res.* 33: 137–143.

MISTRY, V.V. 2001. Low fat cheese technology. *Int. Dairy J.* 11: 413–422.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

MOIOLI, B., PILLA, F. and TRIPALDI, C. 1998. Detection of milk protein genetic polymorphisms in order to improve dairy traits in sheep and goats: a review. *Small Rumin. Res.* 27: 185–195.

MOODY, E.G., VAN SOEST, P.I., MCDOWELL, R.E. and FORD, G.L. 1967. Effect of high temperature and dietary fat on performance of lactating cows. *J. Dairy Sci.* 50: 1909–1916.

MORAND-FEHR, P. and FLAMANT, J.C. 1983. Charactéristiques des laits de brébis et de chèvres. *E.A.A.P. International Symposium on Production of Sheep and Goat in Mediterranean Area*, Ankara, Turkey, p. 384.

MORAND-FEHR, P. and SAUVANT, D. 1980. Composition and yield of goat milk as affected by nutritional manipulation. *J. Dairy Sci.* 63: 1671–1680.

MOTOKI, M. and KUMAZAWA, Y. 2000, Recent research trends in transglutaminase technology for food processing. Food Science Technology Research 6: 151–160.

MOTOKI, M. and SEGURO, K. 1998. Transglutaminase and its use for food processing. *Trends Food Sci. Technol.* 9: 204–210.

MOWLEM, A. 1988. *Goat Farming*. Farming Press, Ipswich, UK.

MOZZON, M., FREGA, N.G., FRONTE, B. and TOCCHINI, M. 2002. Effect of dietary fish oil supplements on levels of *n*-3 polyunsaturated fatty acids, *trans* acids and conjugated linoleic acid in ewe milk. *Food Tech. Biotech.* 40: 213–219.

NIO, N., MOTOKI, M. and TAKINAMI, K. 1985. Gelation of casein and soybean globulins by transglutaminase. *Agric. Biol. Chem.* 49: 2283–2286.

NUDDA, A.M., MCGUIRE, M.A., BATTACONE, G. and PULINA, G. 2005. Seasonal variation in conjugated linoleic acid and vaccenic acid in milk fat of sheep and its transfer to cheese and Ricotta. *J. Dairy Sci.* 88: 1311–1319.

O'CONNOR, P. and FOX, P.F. 1973. Temperature dependent dissociation of casein micelles from the milk of various species. *Neth. Milk Dairy J.* 27(213): 199–127.

O'SULLIVAN, M.M., LORENZEN, P.C., O'CONNELL, J.E., KELLY, A.L., SCHLIMME, E. and FOX, P.F. 2001. Short communication: Influence of transglutaminase on the heat stability of milk. *J. Dairy Sci.* 84: 1331–1334.

OTT, R.S., NELSON, D.R. and HIXON, J.E. 1980. Effect of presence of a male on the initiation of oestrus cycle activity of goats. *Theriogenology* 13: 183–190.

PARIZA, M.W., PARK, Y., COOK, M., ALBRIGHT, K. and LIU, W. 1996. Conjugated linoleic acid (CLA) reduces body fat. *FASEB J.* 10: 3227.

PARK, Y.W. 1990. Nutrient profiles of commercial goat milk cheeses manufactured in the United States. *J. Dairy Sci.* 73: 3059–3067.

PARK, Y.W. 1992. Advances in manufacture of goat cheese. *Proc. V Intl Conf. Goats*, New Delhi, Vol. II, Part II: 382.

PARK, Y.W. 1994a. Basic nutrient and mineral composition of commercial goat milk yogurt produced in the U.S. *Small Rumin. Res.* 13: 63–70.

PARK, Y.W. 1994b. Hypo-allergenic and therapeutic significance of goat milk. *Small Rumin. Res.* 14: 151.

PARK, Y.W. 2000. Comparison of mineral and cholesterol composition of different commercial goat milk products manufactured in USA. *Small Rumin. Res.* 37: 115–124.

PARK, Y.W. 2001. Proteolysis and lipolysis of goat milk cheese. *J. Dairy Sci.* 84(E. Suppl.): E84–E92.

PARK, Y.W. and DRAKE, M.A. 2005. Effect of 3 months frozen-storage on organic acid contents and sensory properties, and their correlations in soft goat milk cheese. *Small Rumin. Res.* 58: 291–298.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

PARK, Y.W. and GUO, M.R. 2006. Goat milk products: Processing technology, types and consumption trends. In: *Handbook of Milk of Non-Bovine Mammals*. Y.W. Park and G.F.W. Haenlein, eds. Blackwell, Oxford and Ames, IA, pp. 59–106.

PARK, Y.W. and HAENLEIN, G.F.W. 2007. Goat milk, its products and nutrition. In: *Handbook of Food Products Manufacturing*. Y.H. Hui, ed. John Wiley & Sons, New York, pp. 447–486.

PARK, Y.W. and HUMPHREY, R.D. 1986. Bacterial cell counts in goat milk and their correlations with somatic cell counts, percent fat, and protein. *J. Dairy Sci.* 69: 32–37.

PARK, Y.W., KALANTARI, A. and VAN HEKKEN, D.L. 2002. Effects of frozen and fresh storage on shelf-life of soft goat milk cheeses. *2002 IFT Abstract* 15B-17.

PARKASH, S. and JENNESS, R. 1968. The composition and characteristics of goat's milk: a review. *Dairy Sci. Abstr.* 30: 67.

PARODI, P.W. 1994. Conjugated linoleic acid: an anticarcinogenic fatty acid present in milk fat. *Aust. J. Dairy Technol.* 49: 93–97.

PATTON, S., LONG, C. and SOKKA, T. 1980. Effect of storing milk on cholesterol and phospholipid of skim milk. *J. Dairy Sci.* 63: 697.

PERRY, D.B., MCMAHON, D.J. and OBERG, C.J. 1998. Manufacture of low fat Mozzarella cheese using exopolysaccharide-producing starter cultures. *J. Dairy Sci.* 81: 563–566.

PETERS, R.R. 1990. Proper milk handling. *Dairy Goat J.* 68(4): 223–227.

PIERRE, A. 1978. Storage of goat milk, intended for the cheese factory in the form of ultrafiltered milk. *Proc. XX International Dairy Congress E*: 788.

PORTMANN, A. 1969. Freezing and storage of goats' milk cheese. Economic importance and quality effects. *Revugen. Frotd Ind. Frigor.* 60: 583; *Dairy Sci. Abstr.* 32: 54(*fide*).

POSATI, L.P. and ORR, M.L. 1976. *Composition of Foods. Dairy and Egg Products. Raw, Processed, Prepared*. Agriculture Handbook No. 8-1. ARS, USDA, Washington, DC.

RAMOS, M. and JUÀREZ, M. 1981. The composition of ewe's and goat's milk. *Int. Dairy Fed. Bull.* Doc. 140.

RAMUNNO, L., RANDO, A., DI GREGORIO, P., MASSARI, M., BLASSI, M. and MASINA, P. 1991. Structura genetica di alcune popuoazioni caprine allevate in Italian a locus della caseina $\alpha_{s1}$. *Proc. IX Congr. Naz. ASPA*, Milan, p. 579.

RAYNAL-LJUTOVAC, K., PIRISI, A., DE CREMOUX, R. and CONZALO, C. 2007. Somatic cells of goat and sheep milk : Analytical, sanitary, productive and technological aspects. *Small Rum. Res.* 68: 126–144.

REMEUF, F. 1992. Physico-chemical properties of goat milk in relation to processing characteristics. *Nat. Symp. Dairy Goat Prod. Marketing*, Oklahoma City, OK. 12–15 August 1992, pp. 98–111.

REMEUF, F. 1993. Effect of $\alpha_{s1}$-casein polymorphism on the properties of goat milk. *Lait* 73: 549–558.

RENNER, E. 1982. *Milch und Milchprodukte in der Ernährung des Menschen*. Volkswirtschaftlicher Verlag, Munich, Germany.

ROGERS, S.A. and MITCHELL, G.E. 1994. The relationship between somatic cell counts, composition and manufacturing properties of bulk milk. 6. Cheddar cheese and skim milk yoghurt. *Aust. J. Dairy Technol.* 49: 70–74.

RUBINO, R. and CLAPS, S. 1995. Goat husbandry systems in Southern Italy. In: *Goat Production Systems in the Mediterranean*. A. El Aich, S. Landau, A. Bourbouze,

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

R. Rubino and P. Morand-Fehr, eds. Wageningen Pers, Wageningen, The Netherlands, EAAP Publ. 71, pp. 68–81.

SCHMIDT, G.H. 1971. *Biology of Lactation*. W.H. Freeman, San Francisco, CA, pp. 178–198.

SHIPE, W.F., BASSETTE, R., DEANE, D.D., DUNKLEY, W.L., HAMMOND, E.G., HARPER, W.J., KLEYN, D.H., MORGAN, M.E., NELSON, J.H. and SCANLAN, R.A. 1978. Off flavors of milk: Nomenclature, standards, and bibliography. *J. Dairy Sci.* 61: 855–869.

STANTON, C., MURPHY, J., MCGRATH, E. and DEVERY, R. 2003. Animal feeding strategies for conjugated linoleic acid enrichment of milk. In: *Advances in Conjugated Linoleic Acid Research, Volume 2*. J.L. Sébédio, W.W. Christie and R. Adlof, eds. AOCS Press, Champaign, IL, pp. 123–145.

STORRY, J.E., GRANDISON, A.S., MILLIARD, D., OWEN, A.J. and FORD, G.D. 1983. Chemical composition and coagulating properties of renneted milks from different breeds and species of ruminant. *J. Dairy Res.* 50: 215–229.

TAMIME, A.Y. and ROBINSON, R.K. 1999. *Yoghurt: Science and Technology*, 2nd edn. Woodhead Publishing, Cambridge, pp. 10–311.

TZIBOULA, A. 1997. Casein diversity in caprine milk and its relation to technological properties: Heat stability. *Int. J. Dairy Technol.* 50: 134–138.

TZIBOULA-CLARKE, A. 2003. Goat milk. In: *Encyclopedia of Dairy Sciences*. H. Roginski, J. Fuquay and P. Fox, eds. Academic Press, London, pp. 1270–1279.

VASSAL, L. and DELACROIX-BUCHET, D. 1994. Effect of AA, EE and FF $\alpha_{s1}$-casein variants on goat cheese production and quality: preliminary results. *Lait* 74: 89–103.

VIGNON, B. 1976. La fraction azotée non protéique du lait, importance, nature et variations. Thesis, Dept. Sci., Univ. Nancy, France.

WAITE, R. and BLACKBURN, P.S. 1957. The chemical composition and the cell count of milk. *J. Dairy Res.* 24: 328–339.

WALSTRA, P., GEURTS, T.J., NOOMEN, A., JELLEMA, A. and VAN BOEKEL, M.A.J.S. 1999. In: *Dairy Technology: Principles of Milk Properties and Processes*. Marcel Dekker, New York, pp. 27–147.

WHITE, C.H. 1995. Manufacture of high quality yogurt. *Cultured Dairy Products Journal* 30(2): 18–26.

YASTGAARD, O.M., NATVIG, H., SWENSON, A. and WILHELMSEN, L.H. 1968. Jernberiking av brunost. *Meierposten* 57(19): 365–375.

ZADOW, J.G., HARDHAM, J.F., KOCAK, H.R. and MAYES, J.J. 1983. The stability of goat's milk to UHT processing. *Aust. J. Dairy Technol.* 38(3): 20–23.

ZHANG, D. and MAHONEY, A.W. 1991. Iron fortification of process Cheddar cheese. *J. Dairy Sci.* 74: 353–358.

ZHU, Y., RINZEMA, A., TRAMPER, J. and BOL, J. 1995. Microbial transglutaminase – A review of its production and application in food processing. *Appl. Microbiol. Biotechnol.* 44: 277–282.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

# 13

# Improving the quality and safety of sheep milk

**R. Bencini, The University of Western Australia, Australia and A. Stanislao Atzori, A. Nudda, G. Battacone and G. Pulina, Università degli Studi di Sassari, Italy**

IP Address: 129.132.208.100

**Abstract:** In this chapter we review the factors that affect the quality of sheep milk and examine the strategies that farmers can adopt to improve the quantity and quality of milk produced by their sheep. Some of these factors, such as the genotype of the sheep, are difficult to control, but farmers have control over others, such as the nutrition and management of the milking sheep. To obtain high quality milk the sheep must be milked regularly and completely, which implies adopting appropriate milking and cleaning routines and milking equipment. It is also important that the sheep are healthy and that they receive adequate diets. Environmental contaminants that potentially make the milk unsuitable for processing should also be avoided. These factors and their influence on milk quality for the processing of milk into dairy products, especially cheese, are described and critically examined.

**Key words:** sheep milk, milk composition, milk quality, processing performance of milk, cheese.

## 13.1 Introduction: a historical perspective

Sheep and goats were domesticated before cattle, and people started to milk them soon after domestication (Clutton-Brock 1981, Anifantakis 1990). We know that sheep were milked and cheese was made from their milk since the Neolithic and the Bronze Age, as prehistoric milk boilers were found in Central Italy together with bones of sheep and goats (De Bellis 1982, Helmer and Vigne

© Woodhead Publishing Limited, 2010

2007), as well as direct evidence of milk residues in pottery found at archeological sites (Dudd and Evershed 1998). The ancient Greeks and Romans also milked sheep and produced cheeses from the milk. We know this from descriptions of cheese-making by Homer (*circa* 700 BC), Columella (*circa* 4 BC–65 AD) and Varro (36 BC). Sheep milking continued in Europe through the Middle Ages as reported by De Bellis (1982).

Sheep milking was practised traditionally in the Mediterranean countries, including Italy, France, Spain, Portugal, Greece, North Africa and some Asian countries. Milking occurred on a seasonal basis, often by keeping the flocks on the mountains in summer and shifting them to the plains in winter, to take advantage of the seasonality of pasture production in these areas. The consequence of this nomadic lifestyle was a low level of production and poor hygiene of the milk (Anifantakis 1990, Boyazoglu and Morand-Fehr 2001).

In these primitive farming conditions, selection towards specialized dairy breeds of sheep was never pushed to the same extent as in dairy cattle (Flamant and Morand-Fehr 1982). As sheep were reared for milking in very poor areas with low pasture production, breeders selected for sheep of low productivity and good adaptation to their environment, as often the available feed was not sufficient to sustain high milk production. It was only after the Second World War that many European governments started encouraging the settlement of shepherds, accompanying this with the selection of sheep for milk production. This was based on progeny testing of rams and resulted in large genetic gains, mainly in France (Flamant and Barillet 1982) and, to a lesser extent, in Spain, Greece and Italy (Bencini 1993, Lindsay and Skerritt 2003).

IP Address: 129.132.208.100

More recently countries that did not have a sheep milking tradition have started milking sheep mainly to satisfy the local demand from ethnic groups. Sheep are milked in Great Britain (Mills 1989), the USA (Thomas 2004), Australia and New Zealand (Bencini 1993). Here genetic gains have occurred by importing specialized breeds of dairy sheep, as progeny testing schemes are hindered by the small size of the industry and consequent lack of organization. This has also created two very different scenarios for the production and processing of sheep milk.

In this chapter we will first review the factors that affect the quality of sheep milk and then we will cover the strategies that can be adopted to improve sheep milk production and quality. As sheep milk is principally transformed into cheese, we will start by examining how the quality of sheep milk is of paramount importance for its processing into high quality cheeses.

## 13.2 Processing of sheep milk

Most of the sheep milk produced in the world is transformed into cheese. Some sheep milk yoghurt is produced in Greece, and fresh sheep milk is consumed rarely. Therefore the quality of sheep milk refers to its capability to be transformed into high quality dairy products, and to produce high yields of these

© Woodhead Publishing Limited, 2010

products from each litre of milk, often described as the processing performance of the milk.

For this reason, the quality of sheep milk is mainly evaluated in terms of its technological and coagulation properties, which are markedly affected by its concentration of fat and protein and somatic cell count (SCC). However, the quality of sheep milk is also affected by the presence and concentration of other components, including those with nutraceutical action such as conjugated linoleic acid (CLA) and other fatty acids (FAs), or those that affect the flavour of milk and its products. However, in all types of cheese, milk protein contributes more than milk fat to total cheese yield (Pulina *et al.* 2006a).

The clotting properties of milk are affected by the composition of the milk (Anifantakis 1986, 1990, Park *et al.* 2007), by the microbiological quality of the milk, by its somatic cell count (Pulina 1990, Kalantzopoulos 1994) and by the cheese-making process itself (Alais 1984).

Cheese makers have some control over the clotting conditions, and can vary pH to achieve the desired acidity by varying the percentage of inoculum of starter cultures (Cogan and Hill 1993). They can also standardize the fat content (Kalantzopoulos 1993) or increase the amount of soluble calcium in milk by adding calcium chloride (Fox 1993). This is often done with milk that does not clot (Alais 1984). Cheese makers also control the temperature and the rennet concentration at which the clotting takes place. However, cheese makers have little or no control over the composition and quality of the milk that reaches the cheese factory. This should be of high microbiological quality, free of antibiotics and have a composition within acceptable limits, but this does not always occur, despite the fact that often the payment for the milk is based on its quality (Pulina 1990).

High protein, fat and total solids concentrations in the milk are associated with high yields in the resulting dairy products. As a consequence, the milk of sheep has a higher yield of dairy products than the milk of cows and goats because it has higher concentrations of protein, fat and total solids (Anifantakis 1986, 1990).

Therefore factors that affect the composition of the milk will affect the yield and quality of dairy products obtained from the milk.

IP Address: 129.132.208.100

## 13.3 Factors affecting the quality of sheep milk

As discussed above, the cheese maker at the cheese factory receives milk of a certain composition and can do very little to change it. The farmer, by contrast, has some control over the environmental factors that affect the quality of sheep milk. These are discussed below.

### 13.3.1 Genetic factors

The breed and genotype of sheep can affect the quality of the milk produced. Selection for dairy production has led to the creation of dairy breeds of sheep that produce more milk than meat and wool sheep. For instance, the Awassi

© Woodhead Publishing Limited, 2010

dairy type can produce up to 1000 litres of milk per lactation (Epstein 1985), but the Poll Dorset, a meat breed, produces only 100–150 litres of milk per lactation (Geenty and Davison 1982).

There is a negative correlation between milk yield and milk composition, so that when animals produce more, the milk usually has a lower concentration of fat and protein (Bencini and Pulina 1997, Table 13.1). This relationship applies not only to the more productive breeds when compared with the less productive (Flamant and Morand-Fehr 1982, Casu and Sanna 1990), but also, within a flock, to those animals that produce more milk (Barillet *et al.* 1986), and within the same animal producing at different levels throughout its lactation (Casoli *et al.* 1989, Pulina 1990). This is generally attributed to the fact that milk volume is determined by lactose secretion, and in highly productive dairy animals the synthesis of fat and protein does not keep up with that of lactose when high rates of milk secretion are achieved (Bencini and Pulina 1997, Pulina *et al.* 2006a). As a consequence, with high milk production, the total amount of cheese produced from the milk can be higher, but the relative yield of cheese from each litre of milk will be lower.

**Table 13.1** Concentrations (g/100 g) of protein and fat in different breeds of sheep

| Breed | Protein | Fat | Source |
|---|---|---|---|
| Awassi | 6.05 | 6.70 | Mavrogenis and Louca (1980) |
| Boutsiko | 6.04 | 7.68 | Voutsinas *et al*. (1988) |
| Bulgaria population | 5.83 | 8.10 | Baltadjieva *et al*. (1982) |
| Chios | 6.00 | 6.60 | Mavrogenis and Louca (1980) |
| Clun Forest | 5.90 | 5.80 | Poulton and Ashton (1970) |
| Comisana | 7.30 | 9.10 | Muscio *et al*. (1987) |
| Dorset | 6.50 | 6.10 | Sakul and Boyland (1992) |
| East Friesian | 6.21 | 6.64 | Shalichev and Tanev (1967) |
| East Friesian cross | 4.98 | 5.00 | Thomas *et al.* (1998) |
| Egyptian population | 5.84 | 8.30 | Askar *et al*. (1984) |
| Epirus | 6.56 | 7.85 | Simos *et al*. (1996) |
| Fat-tailed | 6.40 | 6.26 | Mavrogenis and Louca (1980) |
| Finn | 5.40 | 6.00 | Sakul and Boyland (1992) |
| Karagouniki | 6.60 | 8.70 | Anifantakis (1986) |
| Karakul | 5.57 | 7.36 | Kirichenko and Popov (1974) |
| Lacaune | 5.81 | 7.14 | Delacroix-Buchet *et al*. (1994) |
| Massese | 5.48 | 6.79 | Casoli *et al*. (1989) |
| Merino | 4.85 | 8.48 | Bencini and Purvis (1990) |
| New Zealand Romney | 5.50 | 5.30 | Barnicoat (1952) |
| Rambouillet | 5.90 | 6.10 | Sakul and Boyland (1992) |
| Romanov | 6.10 | 5.90 | Sakul and Boyland (1992) |
| Sarda | 5.89 | 6.61 | ARA (1995) |
| Suffolk | 5.80 | 6.60 | Sakul and Boyland (1992) |
| Sumava | 6.47 | 7.93 | Flam *et al*. (1970) |
| Targhee | 4.51 | 9.05 | Reynolds and Brown (1991) |
| Tzigai | 5.45 | 7.41 | Margetin (1994) |
| Vlachiki | 6.52 | 9.05 | Anifantakis (1986) |
| Welsh Mountain | 5.40 | 6.20 | Owen (1957) |

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

The genotype of the sheep may also affect the clotting properties of the milk through different genetic variants of the casein fractions (McLean *et al.* 1984, 1987). For dairy cows, the presence of certain casein genotypes can affect the composition of the milk (Aleandri *et al.* 1990), and Italian researchers have identified individual cows carrying particular genetic variants of the $\kappa$-casein which make the milk unsuitable for Parmesan cheese making due to poor coagulation (Losi and Mariani 1984).

In European dairy sheep, polymorphism of the casein has been reported by Lyster (1972), Arave *et al.* (1973), Davoli *et al.* (1985), Russo *et al.* (1981), Chiofalo *et al.* (1982), Bolla *et al.* (1985, 1986) and Manfredini *et al.* (1987). The $\alpha_{s1}$ casein variant named 'Welsh', the frequency of which varies from 2.2% (Chiofalo and Micari 1987) to 22% (Caroli *et al.* 1989), causes a reduction in casein content and a worsening of milk clotting properties in homozygous animals and, to a lesser extent, in heterozygous animals (Piredda *et al*. 1993). In Australian sheep, Thomas *et al.* (1989) and Clément *et al.* (2006) reported the existence of genetic variants of the casein, but it is still not clear whether these genetic variants have an effect on clotting properties and cheese outcome.

Few studies have been conducted on renneting times, coagulation patterns, rates of curd formation and curd firmness of sheep milk (Askar *et al.* 1984, Manfredini *et al.* 1987, Casoli *et al.* 1992, Delacroix-Buchet *et al.* 1994, Bencini and Johnston 1996, Bencini 2002). All these studies confirmed that sheep milk has short renneting times, fast rates of curd formation and high consistency of the curd, characteristics that are all associated with high yields of cheese from each litre of milk.

IP Address: 129.132.208.100

Despite the reported differences in composition of the milk between different breeds of sheep, Italian researchers have shown that there was no difference in cheese-making performance of milk between the breeds Sarda, Comisana, Massese and Delle Langhe (Chiofalo *et al.* 1989, Casoli *et al.* 1990, Ubertalle *et al.* 1991).

In France, Delacroix-Buchet *et al.* (1994) calculated the coefficients of genetic repeatability in the Lacaune breed for renneting time (0.57), rate of curd formation (0.48) and curd consistency (0.53) and concluded that, since these repeatability values are similar to the repeatability for milk production (*circa* 0.5), family type selection would be needed to achieve genetic progress.

### 13.3.2 Somatic cell count

The health of the sheep, and especially that of the mammary glands, affects the quantity and the quality of the milk produced. Mastitis is the most common pathology of the mammary gland in sheep dairies and is economically important because it reduces milk production and causes qualitative changes in milk composition, which alter the processing performance of the milk and the qualitative characteristics of the dairy products derived from it. This is due to a decreased synthetic capacity of the mammary secretory cells and to an increased permeability of the mammary epithelium that cause the passage of blood com-

© Woodhead Publishing Limited, 2010

ponents directly into the milk and an increase in the somatic cell count of the milk (Morgante *et al.* 1994). As a consequence, the milk of dairy sheep suffering from mastitis does not clot and is not suitable for cheese production (Casoli *et al.* 1992).

### 13.3.3 Microbial cell count

The microbial cell count in milk is due to the presence of microorganisms of which some (*Lactobacillus* spp., *Lactococcus* spp., *Streptococcus* spp.) can be advantageous for transformation into cheeses, while others can cause human diseases (e.g. *Listeria*, *Salmonella*, *Brucella*) or problems in the maturation of the dairy products (e.g. Enterobacteriaceae, coliforms, psychrotrophs, *Clostridium* spp.; Fatichenti and Farris 1973).

Psychrotrophic bacteria such as *Pseudomonas*, *Leuconostoc* and *Micrococcus* thrive at temperatures below 7ºC and produce lipolytic and proteolytic enzymes which destabilize the casein micelles and alter the clotting properties of milk (Nuñez *et al.* 1989, Uceda *et al.* 1994a, 1994b).

Enterobacteriaceae and coliforms are generally of faecal origin and ferment the lactose in the cheeses, producing large quantities of gas, causing early spoilage of the cheese (Gaya *et al.* 1987). The occurrence of undesirable bacteria can be avoided by applying correct milking and milk handling procedures.

Health regulations often prescribe the maximum bacterial and somatic cell counts allowable for the processing of sheep milk. These regulations vary for different countries and in some countries all dairy products must be produced from pasteurized milk. This is not strictly required in the Mediterranean countries, where some cheeses are produced from raw milk.

IP Address: 129.132.208.100

## 13.4 Physiological factors affecting the quality of sheep milk

### 13.4.1 Age and parity

Maiden ewes produce less milk than older ewes and maximum yields are generally achieved at the third or fourth lactation, after which total lactation yields tend to decrease (Bencini and Pulina 1997). As these order-of-parity factors are often confounded with the age of the ewes, it is impossible to distinguish between the two. The parity of the ewes affects milk composition: with increasing lactation number the milk contains higher concentrations of fat and protein, higher somatic cell counts and lower concentrations of lactose (Bencini and Pulina 1997).

Since the negative relationship between yield and milk quality has been confirmed to apply to individual animals within a flock (Barillet *et al.* 1986), changes in milk yield brought about by age and lactation number are likely to result in changes in the composition of the milk as reported by Pulina (1990) and Pulina *et al.* (1992, 2006a).

© Woodhead Publishing Limited, 2010

### 13.4.2 Stage of lactation

The stage of lactation markedly affects the amount of milk produced. Lactation begins at parturition and daily yields increase rapidly for the first few weeks. Peak yields are achieved around the third to fifth week of lactation (Bencini and Pulina 1997). After the peak, lactation declines more or less rapidly depending on the breed and genotype and on individual dairy potential. The concentrations of fat, protein (both casein and whey protein), total solids and somatic cells are high at the beginning and end of lactation, and low at peak lactation, while the concentration of lactose follows closely the lactation yield (Bencini and Pulina 1997). The mineral content of milk is also affected by the stage of lactation: throughout lactation there is an increase in chloride (Pauselli *et al.* 1992) and magnesium and a reduction in potassium (Polychroniadou and Vafopulou 1984).

The processing performance of the milk also changes with the stage of lactation. As lactation progresses the clotting properties of the milk tend to worsen, with an increase in renneting time and rate of curd formation and a decrease in the consistency of the curd (Ubertalle 1989, 1990).

### 13.4.3 Weight of the ewes

Most authors agree that heavier ewes produce more milk (Burris and Baugus 1955, Owen 1957, Boyazoglu 1963); however, there are few reports on the effect of liveweight on the quality of the milk for cheese making. Pulina *et al.* (1994a) found positive phenotypic correlations (from 0.26 to 0.56) between the liveweight of Sarda ewes and the concentrations of fat and protein of their milk for the first 10 weeks of lactation.

IP Address: 129.132.208.100

### 13.4.4 Number of lambs born or weaned

Sheep suckling twin lambs produce more milk than those suckling single lambs, and ewes rearing triplets produce more milk than those rearing twins (Bencini and Pulina 1997). However, there are few and contradictory reports on the effect of number of lambs reared or born on the quality of milk. Gardner and Hogue (1964) reported that Rambouillet and Columbia ewes rearing single lambs had a lower concentration of fat in their milk than ewes rearing twins. By contrast, in a later study, these authors reported that Hampshire and Corriedale ewes that gave birth to single lambs produced milk with a higher concentration of fat and protein (Gardner and Hogue 1966). This was also reported by Serra *et al.* (1993) who observed that Sarda ewes that gave birth to single lambs produced milk with a higher concentration of fat and protein throughout lactation, but their milk production was lower than that of sheep with twin lambs. The negative relationship between yield and quality of milk may explain why twin-bearing ewes that produce more milk have lower concentrations of fat and protein in the milk. The contrasting result reported by Gardner and Hogue (1964) may be due to the small number of ewes (10 single- and 10 twin-bearing ewes) used in their experiment.

© Woodhead Publishing Limited, 2010

Milk yield is greater in ewes bearing multiple lambs even if they do not nurse them (Pollot and Gootwine 2004) but if dairy ewes are allowed to suckle their lambs for the first few weeks of lactation the yields of milk from ewes that reared twin lambs should be, at least initially, higher than those of ewes rearing singles. For this reason in the Middle East the ewes are allowed to suckle their lambs after the evening milking (Folman *et al.* 1966, Morag *et al.* 1970, Eyal *et al.* 1978). This weaning method affects the composition of the milk because sheep that are nursing their lambs do not have a normal milk ejection reflex (Bencini 1999, Marnet and McKusick 2001, McKusick *et al.* 2002) and some sheep milk producers advocate the destruction of newborn lambs to simplify the management of the milking operations. However, sheep that are allowed to feed their lambs produce more milk and have longer and more persistent lactations, resulting in a greater total lactation yield (Bencini 1999). In support of this, Ubertalle (1990) reported that early weaning of the lambs worsened the consistency of the curd derived from the milk even though the composition of the milk was not affected. This is probably due to the fact that oxytocin and prolactin that normally prevent mammary involution are reduced if lambs are removed early (Turner and Huynh 1991) and their reduction results in a reduction of mammary DNA and an increase of plasminogen activators. These convert plasminogen into plasmin, which is involved in the hydrolysis of $\beta$-casein, so that the final consistency of the curd is reduced.

IP Address: 129.132.208.100

## 13.5 Management factors affecting the quality of sheep milk

### 13.5.1 Milking techniques

In the Mediterranean countries sheep are occasionally still hand-milked with the consequence of poor hygiene and high bacterial counts in the milk (Anifantakis 1990). However, the concentration of protein and fat in the milk does not differ between hand and machine-milked ewes (Casu *et al.* 1978a).

In countries where sheep milking has been introduced recently, sheep are milked by machine and the milk has better microbiological characteristics. The machine-milking of sheep has been reviewed by Purroy Unanua (1986). The design of milking parlours and the organization of the milking operations have been described by Enne (1976) and Pazzona (1980) in Italian and by Kervina *et al.* (1981), Mills (1989) and Stubbs *et al.* (2009) in English. The practical recommendations of Stubbs *et al.* (2009) are particularly relevant to the emerging sheep-milking industries of Australia, New Zealand and, to a lesser extent, the USA.

### 13.5.2 Milking interval and frequency

As the rate of milk secretion is controlled locally by the feedback inhibitor of lactation (FIL), a fraction of the whey proteins present in milk (Wilde and Peaker 1990, Wilde *et al.* 1996), the interval and frequency of milking assume

© Woodhead Publishing Limited, 2010

paramount importance in affecting the yield of milk. The findings of Denamur and Martinet (1961) and Grigorov and Shalichev (1962) provided some indirect evidence that an autocrine control of milk secretion is present in sheep. This was later confirmed by Bencini *et al.* (2003). Therefore, the interval between milking, the milking frequency and the adoption of stripping methods to remove additional milk and ensure completeness of milking increase both the daily output of milk and the total lactation yield of dairy ewes by removing the inhibitory effect of milk accumulated in the alveolar tissue of the mammary glands.

In dairy sheep, reducing the frequency of milking results in a loss in milk production (Morag 1968, Karam *et al.* 1971, Bencini *et al.* 2003), but there are few and contrasting reports on the effect of milking frequency and interval on the composition of the milk. Casu and Labussière (1972) and Huidobro (1989) reported that omitting one or both of the milkings on a particular day of the week did not affect the composition of the milk. When the milking frequency was reduced from twice to once daily the composition of the milk was not affected in the studies of Casu and Boyazoglu (1974), De Maria-Ghionna *et al.* (1982) and Cannas *et al.* (1991), but fat and protein concentrations were increased in the studies of Battaglini and De Maria (1977) and Battaglini *et al.* (1977, 1979), and reduced in a study by Morag (1968). When the milking frequency was increased from two to three times daily, milk composition was not affected according to Morag (1968) and Cannas *et al.* (1991), but the concentrations of fat and protein were increased according to Mikus and Masar (1978).

Such contrasting reports may be due to the fact that these studies were conducted with different breeds of sheep, which would have efficient or inefficient autocrine control of milk secretion according to the degree of selection for dairy production. Bencini *et al.* (2003) reported that milk composition was affected by the milking interval and frequency in breeds that are not selected for dairy production because they have an efficient control of milk secretion. On the contrary, breeds selected for dairying have an impaired autocrine control mechanism and therefore do not respond as much to changes in milking frequency (Nudda *et al.* 2002).

IP Address: 129.132.208.100

### 13.5.3 Stripping method

Labussière (1988) showed that European sheep had two different kinds of milk ejection patterns: some ewes released their milk in a single peak and others had a delayed let-down, so that two distinct peaks were observed in the milk release curve. Double-peaked sheep are present in a greater proportion within the specialized dairy breeds. Labussière *et al.* (1969) studied the milk ejection reflex in Préalpes ewes and concluded that the second peak of milk ejection occurred because the ewes released oxytocin, which resulted in milk ejection. Labussière (1969) and Ricordeau (1974) observed that the milk obtained from double-peaked animals had a higher fat concentration and concluded that the double peak was due to a delayed release of endogenous oxytocin, so that the first peak

© Woodhead Publishing Limited, 2010

corresponded to the removal of the cisternal milk and the second peak, within a minute of the first, corresponded to the alveolar milk ejected due to the release of endogenous oxytocin. This is in agreement with Ranieri (1993) who measured the composition of milk fractions collected during milking and observed a progressive increase in the concentration of fat, but no changes in the concentrations of protein and lactose as milking proceeded. Mayer (1990) measured plasma oxytocin concentrations in single- and double-peaked ewes and confirmed the conclusions of Labussière (1988) and Purroy Unanua (1986), that ewes with a double peak had an effective ejection reflex, which allowed for a better removal of milk from the mammary glands. By contrast, ewes with a single peak of milk ejection did not release oxytocin so that only the cisternal milk, which has a low concentration of fat, was collected.

The fact that animals with single peaks have high residual milk, shorter lactations (Labussière 1985, 1988, Purroy Unanua 1986) and lower concentration of fat in the milk (Mikus 1970) supports the adoption of practices to increase the removal of milk from the mammary glands. Labussière (1985, 1988) and Bencini and Knight (1994) demonstrated that stripping by hand or by machine is necessary for unselected sheep that have only one peak of milk ejection.

However, for Sarda sheep with a double peak of ejection, Casu *et al.* (1978b) reported that double cupping can be eliminated without affecting the composition of the milk, and Purroy Unanua (1986) reported that manual stripping can be replaced successfully with machine stripping, or double cupping, without compromising the amount of milk withdrawn. Additionally, McKusick *et al.* (2003) found that when machine stripping was eliminated in highly selected East Friesian sheep the 14% loss in milk yield was compensated by a greater throughput of sheep.

IP Address: 129.132.208.100

### 13.5.4 Environmental conditions and milk composition

Air temperature is a key microclimatic parameter for the well-being of sheep and is affected by airflow and humidity. Exposure of sheep to temperatures above 30ºC depressed the immune response and decreased milk production as well the casein and fat concentration of the milk, and worsened the hygiene and quality of milk with an increase in neutrophiles, staphylococci, coliforms and Pseudomonadaceae in the milk. This was accompanied by a worsening of the processing performance of the milk, due in part to a lower concentration of calcium, phosphorus and casein, and in part to the increased activity of plasmin, which is the main proteolytic endogenous enzyme in milk (Sevi *et al.* 2001a),

Air quality is also important for sheep housed indoors. Carbon dioxide, ammonia, sulphide and methane are the main gases detected in increased levels in animal houses. A low ventilation regimen (33 $m^3$/h per ewe) caused a significant reduction in milk yield, depressed immune reactivity, and increased plasma cortisol in ewes during summer. While casein concentration was lower in the milk of the sheep kept under the low ventilation regime, clotting time, curd firmness, pH and SCC of milk were unaffected (Sevi *et al.* 2001b).

© Woodhead Publishing Limited, 2010

Sevi *et al.* (2001b) also found that total microbial count and coliforms were lower in the air if sheep had 2 $m^2$ of space each compared to sheep that had only 1.5 $m^2$ or 1.0 $m^2$. The sheep also produced more milk in the less crowded environment, and the milk had a greater concentration of protein, casein and fat.

### 13.5.5 Management of the ewes

Some management practices such as shearing, out-of-season breeding, artificial lighting/photoperiod and the use of hormones may affect the quantity and quality of milk produced.

*Shearing*

Shearing sheep before or immediately after lambing increases the concentration of protein and fat in the milk of Poll Dorset ewes (Knight *et al.* 1993). This may be caused by an increase in feed intake (Wodzicka-Tomaszewska 1964, Vipond *et al.* 1987) leading to an increase in blood glucose (Kirk *et al.* 1984), probably in response to cold stress (Wheeler *et al.* 1962).

Rassu *et al.* (2009) found that milk fat content increased from 6.37% pre-shearing to 6.94% post-shearing. The content of fatty acids C8, C10, C12 and C16 also significantly increased from pre-shearing to post-shearing but without affecting the content of long-chain fatty acids (C > 18:0). This suggests that the effects of shearing on milk composition are not simply due to temperature and that the mechanisms involved in the modification of the FA profile of milk after shearing should be elucidated.

IP Address: 129.132.208.100

*Breeding out of season*

In the Mediterranean countries, sheep milking is seasonal, and even the largest and most modern sheep milk cheese factories are closed during summer.

Sevi *et al.* (2004) observed that the milk yielded by winter-lambing ewes had lower fat and protein contents compared to that of autumn-lambing ewes, as well as worse processing performance and hygienic characteristics. Irrespective of the lambing season, a higher SCC was recorded in late, compared to early and mid-lactation milk (6.16 vs. 5.93 and 5.87 $\log_{10}$ somatic cells/ml) as well as a marked worsening in clotting properties. Reduced cheese-making efficiency was associated with a reduction in the casein and fat content of milk from autumn-lambing ewes, and decreased calcium and phosphorus content in the milk of ewes that lambed in winter. In Europe, sheep milk produced in summer is strongly affected by the combined effects of seasonal changes in climate and herbage availability and variations in the metabolic status of the sheep with the advancement of lactation. For these reasons, the milk has poor cheese-making performance (Delacroix-Buchet *et al.* 1994) due to long renneting times, poor consistency of the curd and high proteolytic and lipolytic activities.

Jaeggi *et al.* (2005) observed a decrease in total solids in the milk collected in winter, late spring and summer as the season progressed and attributed this to the hot weather and poorer quality pastures grazed by the animals in summer. As a

© Woodhead Publishing Limited, 2010

result, cheese yield was higher for milk collected in winter than for milk collected in spring and summer. Thompson *et al.* (1982) reported that high temperatures do not have an effect on the composition of sheep milk, but long day lengths result in lower protein concentrations in the milk (Bocquier *et al.* 1990) and in reduced rates of secretion of fat and protein (Pulina *et al.* 1994b). In summer, strategies to reduce the impact of high temperatures on lactating ewes and supplementation with minerals are necessary to sustain the yield and quality of milk and cheese (Sevi *et al*. 2004).

The high variability of milk quality in summer may be attributed to the poor nutritive value of pasture, as Pulina *et al.* (1993) demonstrated that a balanced ration restored the cheese-making performance of summer milk. In order to improve the efficiency of cheese factories and exploit the greater market demand during the summer period, Piras *et al.* (2007) proposed a lambing plan to produce milk also in summer and early autumn, using irrigated pastures, resulting in yields and quality of milk that were very comparable to milk produced in the traditional system.

When sheep dairying was first established in Australia and New Zealand, most farmers opted for a year-round supply of milk, which was made possible by the fact that the local sheep were less seasonal than the European breeds, and if appropriately managed, could be bred out of season (Scaramuzzi and Martin 1984, Signoret 1990). However, sheep dairy farmers experience a number of problems connected to the out-of-season breeding of their dairy flocks. First, only a proportion of ewes will mate out of season, so most of the lambings are concentrated in winter. Second, the availability of good pasture during summer is low, especially in Australia, and those sheep that have lambed tend to produce less milk. As a consequence severe shortages of milk occur during the summer months, when the demand for fresh sheep milk yoghurt is highest. The differential nutrition also changes the processing performance of the milk and consequently the quality of the derived dairy products. So, it is difficult to maintain a constant and consistent production throughout the year. As breeding out of season is often pursued through the use of hormones, it is also not consistent with the recent trend towards clean, green and ethical animal production that aims at minimizing the use of hormones (Martin *et al.* 2004).

IP Address: 129.132.208.100

*Artificial lighting/photoperiod*

Photoperiod determines the reproductive season in sheep, especially at high latitudes, but it also affects milk production and milk composition. Exposing the sheep to long days increased milk yield but resulted in lower concentrations of fat and protein in the milk (Bocquier *et al.* 1990, Pulina *et al.* 1994b, Chilliard and Bocquier 2000). By contrast, Morrissey *et al.* (2008) found no difference in the composition of the milk. Short day lengths before lambing had a positive effect on milk production in the following lactation, possibly because photoperiod affects progesterone, prolactin and placental lactogen that regulate mammary development during pregnancy (Pollot and Gootwine 2004).

© Woodhead Publishing Limited, 2010

*Use of hormones*

Growth hormone increases milk production in dairy cows (Bauman 1999). This occurs also in dairy sheep, where the increase in milk production is accompanied by increases in milk fat concentration and reductions in milk protein, which is not desirable for cheese making (Fleet *et al.* 1986, 1988, Holcombe *et al.* 1988, Pell *et al.* 1989, Sandles *et al.* 1988, Stelwagen *et al.* 1993). In any case, the use of hormones to increase milk production is not allowed in Europe and Australia.

### 13.5.6 Nutrition

Since nutrition affects both the yield and the composition of the milk produced, it also affects the quantity and quality of cheese (Bencini and Pulina 1997). Several nutritional factors affect milk fat concentration and yield. Among the most important factors are the energy balance (EB) of the ewes, the concentration, intake and source of dietary neutral detergent fibre (NDF) and non-fibrous carbohydrates (NFC), the particle size of feeds and fibre, as well as the amount, physical characteristics and fatty acid composition of dietary fat supplements. Interactions among these factors and other less important ones make prediction of milk fat concentration complex. These will be examined in more detail in the next section, on the improvement of sheep milk.

## 13.6 Improving sheep milk production

IP Address: 129.132.208.100

### 13.6.1 A genetic approach

The extension of dairy sheep breeding goals to milk quality and functional traits should be carefully evaluated both from a technical and economical viewpoint. The classical quantitative selection approach for improving milk composition is difficult due to the negative genetic correlation with milk yield and the high cost of phenotype recording. A selective goal should be to increase the fat and protein yield independently from milk yield to avoid any negative consequences on the fat and protein concentrations (Casu *et al.* 2009).

Aggregate genetic indexes that also consider milk fat and protein contents have been adopted in France for the Lacaune sheep, in order to prevent a possible reduction in milk fat and protein contents caused by selection based on milk yield (Barillet 1997). As almost all sheep milk is processed into cheese, Othmane *et al.* (2002) suggested the use of individual cheese yield that can be recorded on each test day. This new variable, which is correlated to the milk fat and protein contents ($r = 0.52$ and 0.38, respectively), has the advantage of expressing in a single term the suitability of milk for cheese processing.

The increased knowledge on genomic regions controlling quantitative traits of economic interest could be implemented into marker assisted selection schemes for improving breeding value estimation, enhancing selection intensity and reducing generation interval (Cappio-Borlino *et al.* 1996, Russo and Fontanesi 2001). Research into quantitative trait loci (QTLs) is constrained by

© Woodhead Publishing Limited, 2010

the extensive time and resource requirements, mainly due to the large number of animals that have to be genotyped to obtain a reasonable statistical power of the experimental design (Rebai *et al.* 1995, Carta and Elsen 1999). However, research has been carried out on QTL mapping in dairy sheep and interesting results for milk composition have been reported (Table 13.2).

On the other hand, selection for sheep milk quality has to deal with the continuous evolution of the concept of quality, which includes a marked drift toward nutraceutical aspects. A significant example is represented by the recent interest in the milk content of conjugated linoleic acid (CLA). Differences in the genomic region containing the stearoyl Co-A (SCD) gene, which encodes for the delta-9-desaturase enzyme, could affect the CLA content of the milk. The SCD cDNAs of different species, including hamster, rat, mice, human and sheep, have been cloned, but only one SCD gene isoform has been identified in sheep, located on chromosome 22 (Ward *et al.* 1997).

A remarkable variability in CLA content has been detected in a backcross Lacaune × Sarda population: individual variance accounted for 29.7% of the total phenotypic variance for CLA milk content and 34.4% for the CLA/vaccenic acid ratio, while sire variances were smaller (8.4% and 5.6%, respectively, for CLA milk content and the CLA/vaccenic acid ratio). Moreover, a QTL affecting the CLA/vaccenic acid ratio was found on OAR 22 (i.e. on the chromosome where the SCD gene is located; Carta *et al.* 2003). This result suggests a possible relationship between SCD gene polymorphism and its expression level, with a consequent effect on the quantity of CLA produced in the mammary gland from vaccenic acid. These findings open a very interesting scenario for the future implementation of molecular genetics in the selection of milk quality traits.

IP Address: 129.132.208.100

**Table 13.2** Quantitative trait loci (QTLs) reported for dairy sheep

| Trait | Location | Breed | Source |
|---|---|---|---|
| CLA/vaccenic acid in milk fat | OAR22 | Sarda × Lacaune BC | Carta *et al.* (2003) |
| Milk fat content | OAR20 | Sarda × Lacaune BC | Carta *et al*. (2003) |
| Milk protein content | OAR6 | Churra | Diez-Tascon *et al.* (2001) |
| Milk protein content | OAR1 | Sarda × Lacaune BC | Carta *et al*. (2003) |
| Milk yield | OAR3 | Sarda × Lacaune BC | Carta *et al.* (2003) |
| Resistance to nematodes | OAR2, OAR6 | Sarda × Lacaune BC | Moreno *et al*. (2006) |
| Somatic cell score | OAR6 | Sarda × Lacaune BC, Lacaune | Rupp *et al.* (2003) |
| Teat placement | OAR9, OAR14 | Sarda × Lacaune BC | Casu *et al.* (2003) |
| Udder attachment | OAR12 | Sarda × Lacaune BC | Casu *et al*. (2003) |
| Fat yield | OAR7 | Sarda × Lacaune BC | Casu *et al.* (2009) |
| Protein yield | OAR7 | Sarda × Lacaune BC | Casu *et al.* (2009) |

Source: Macciotta *et al.* (2005).

© Woodhead Publishing Limited, 2010

Somatic cell count (SCC) is an important trait indicating the susceptibility of animals to mastitis and it may indirectly improve the quality of the milk. However, indirect selection against mastitis by using SCC as an indicator trait is constrained by the low values of heritability and by its large standard errors, also demonstrated in dairy cattle (Macciotta *et al.* 2005). At present, only the Lacaune breeding programme takes SCC into account. An alternative approach could be the inclusion of SCC in an aggregate genotype that considers other functional traits related to milk production such as udder morphology, speed of milking and persistency of lactation. In particular, udder conformation is a trait that greatly affects the extent of manual intervention needed for extracting the milk retained in the udder (Casu *et al.* 2003). A linear scale for the morphological appraisal of the udder is currently used in Spain and Italy (de la Fuente *et al.* 1996, Casu *et al.* 2002). Genetic parameter estimates highlight a low to moderate genetic variability for the trait (Macciotta *et al.* 2005). Higher values found for the Sarda breed could be due to differences in the model used, particularly in the use of random contemporary groups, and in the size of the population analysed. Other measures, such as the size of the mammary cistern evaluated by ultrasound technique, could also be considered for genetic selection (Nudda *et al.* 2000).

*Genetic engineering*

Transgenic sheep for the growth hormone (GH) gene developed in Australia by the CSIRO produced twice as much milk than non-transgenic controls, but the milk had a lower concentration of protein, including casein (Bencini 2005). As the GH transgenic sheep produced the extra GH naturally, it should have been feasible to develop commercial applications. The transgenic sheep were destroyed due to animal welfare problems and changes in the legislation. Given the current attitude of consumers towards genetically modified organisms, it is unlikely that transgenic technology will be used to improve sheep milk production.

IP Address: 129.132.208.100

### 13.6.2 A non-genetic approach

The fat and protein content of milk from ewes with similar production levels can be improved, or worsened, by means other than genetics.

The variability is higher for fat vs. protein yield, with the total variance accounted for by the allometric model used to fit the data being 82–92% for fat and 92–98% for protein in the Sarda breed (Pulina *et al.* 2006a). Because the relationship between milk fat concentration and yield has a higher variability than that between milk protein concentration and yield (Fig. 13.1), modification of milk composition by non-genetic means should be easier to achieve for fat than for protein.

### 13.6.3 Nutrition

Among the most important nutritional factors affecting milk fat concentration and yield are the energy balance (EB) of the ewes, the concentration, intake and

© Woodhead Publishing Limited, 2010

© Woodhead Publishing Limited, 2010
IP Address: 129.132.208.100

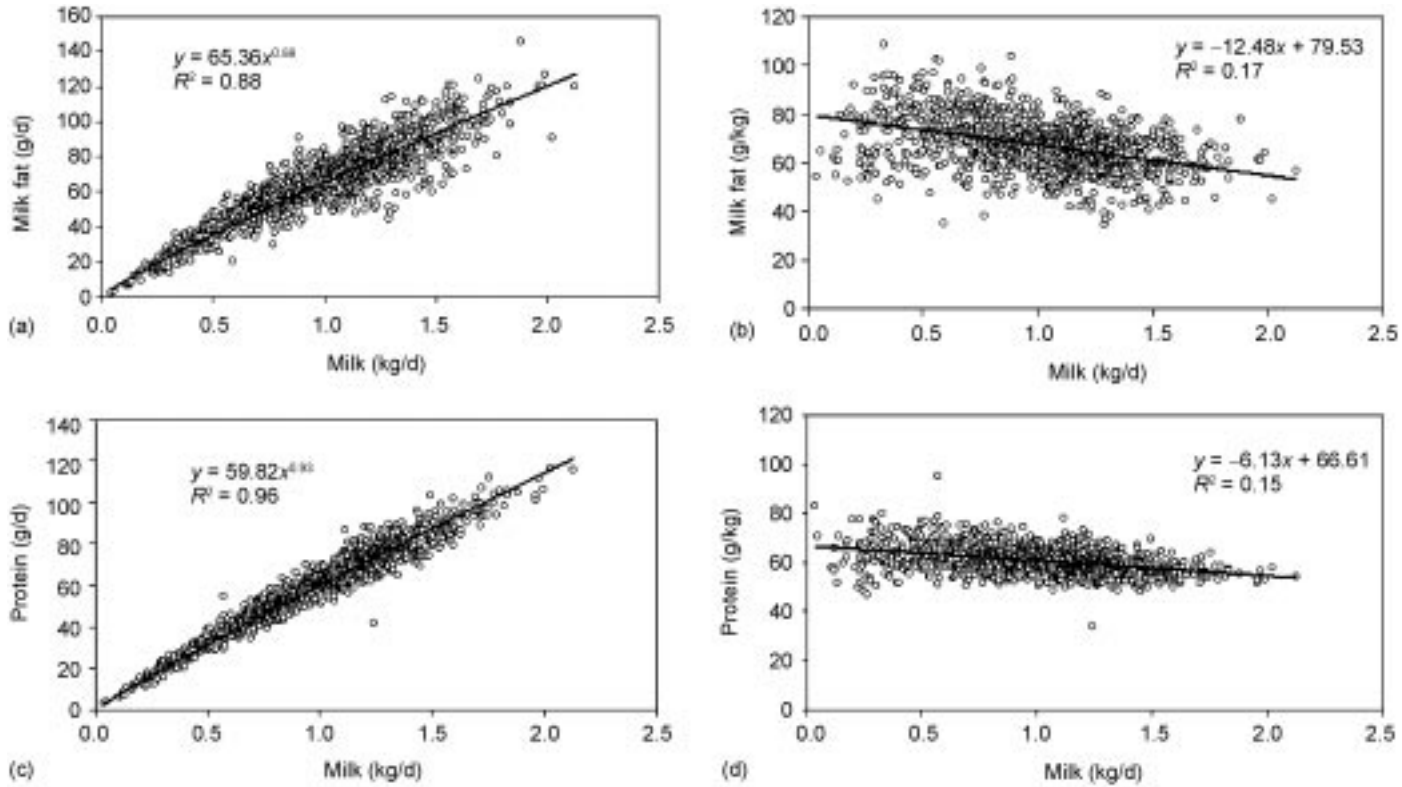



**Fig. 13.1** Relationships between milk yield and (a) milk fat yield, (b) milk fat concentration, (c) milk protein yield and (d) milk protein concentration, based on 1665 individual data of Sarda ewes (adapted from Pulina *et al.*, 2006a).

source of dietary neutral detergent fibre (NDF) and non-fibrous carbohydrates (NFC), the particle size of feeds and fibre, as well as the amount, physical characteristics and FA composition of dietary fat supplements. Interactions among these factors and other less important ones make prediction of milk fat concentration complex.

*Energy balance and milk fat and protein content*

The energy balance (EB) of the sheep is probably the most important factor that affects milk fat concentration, especially in the early stages of lactation. A negative association between EB and milk fat concentration was reported for dairy cows (Palmquist *et al.* 1993) and sheep (Table 13.3). The similarity between the slopes of the two equations in Table 13.3 suggests that the rate at which mobilized body fat contributes to milk fat concentration is similar among breeds. However, the different intercepts suggest differences in basal fat concentration.

When the sheep mobilize body reserves, usually at the beginning of lactation, there is an increase in blood concentrations of long-chain FA (LCFA), derived from the mobilization of body fat triglycerides resulting in an increase of their concentration in the milk fat (Rossi and Pulina 1991). As ewes move from negative to positive EB, generally as lactation progresses, the milk fat LCFA fraction decreases.

The correlation between milk fat concentration and EB is weaker for ewes that have low milk yields, possibly because low-producing sheep are almost always in positive EB (Cannas and Avondo, 2002). Thus, milk fat concentration could be used to predict the EB of sheep or differences in EB in potentially problematic animals, which lose body condition too quickly (de Vries and Veerkamp 2000).

Milk protein concentration in sheep is positively associated with the energy concentration of the diet ($r = 0.64$; Bocquier and Caja 2001). Indeed, ewes with high milk yield fed high-grain and high net energy (NE) diets in early lactation had higher milk protein concentration and yield than ewes fed low-grain and low-energy diets (Susin *et al.* 1995). By contrast, no effects were reported on

IP Address: 129.132.208.100

**Table 13.3** Relationship between daily energy balance (EB, Mcal of $NEL_{1M}$/day) and milk fat content (MF, g/100 ml) in sheep

| Breed | Milk yield (l/day per head) | Fat (g/100 ml) | Equation | $R^2$ | Source |
|---|---|---|---|---|---|
| Dairy and non-dairy breeds | 0.650–3.500 | 4.2–9.8 | $MF = 9.640 - 0.718 \times EB$ | 0.76 | Bocquier and Caja (1993, 2001) |
| Comisana | 0.650–1.600 | 4.0–10.6 | $MF = 6.990 - 0.712 \times EB$ | 0.14 ($P < 0.0001$) | Cannas and Avondo (2002) |

© Woodhead Publishing Limited, 2010

milk protein concentration in ewes fed pelleted diets, at two NE concentrations, in mid-lactation (Cannas *et al.* 1998).

*Dietary fibre*

The well-known association between the content of neutral detergent fibre (NDF) of the diet and milk fat concentration in dairy cows (Emery 1988) is also present in sheep (Table 13.4) and is also supported by the study of Nudda *et al.* (2004a), who reported a positive correlation ($r = +0.38$) between dietary NDF concentration and milk fat concentration. However, different results were observed in sheep grazed outdoors or fed indoors, possibly due to the different feeding regimes. In sheep fed indoors, an increase in NDF concentration caused a reduction of DM intake (DMI), net energy (NE) intake and milk yield, possibly because sheep fed these diets can select very little among the dietary ingredients (Serra 1998). By contrast, in grazing sheep, ewes with higher milk yield eat more pasture (and fibre) to meet their nutrient requirements. As intake of NE increased, due to the increase in pasture and NDF intake, the EB improved, causing a decrease, or interruption, of body fat mobilization. This probably reduced the availability of LCFA to the mammary gland, contributing to a decrease in the synthesis of milk fat (Cannas and Avondo 2002).

*Fat supplements*

Fat supplements can be added to the diet of sheep to increase dietary energy concentration, or to modify the concentration of milk fat, or its FA profile. Fat supplements can be protected or unprotected from rumen microbial activity.

High concentrations of fat added to diets can reduce the rumen fermentation activity of the bacteria and protozoa, thus reducing the advantage of the higher energy density of the diet (Palmquist and Jenkins 1980). Alteration of rumen microbial activity might also reduce milk fat synthesis, reduce the synthesis of

IP Address: 129.132.208.100

**Table 13.4** Relationship between the dietary content of Neutral Detergent Fibre (NDF, % of DM) and milk fat content (MF, g/100 g) in sheep

| Breed | Milk yield (kg/day per head) | Feeding | Equation | $R^2$ | Source |
|---|---|---|---|---|---|
| Sarda | | Indoors | MF = 4.59 + 0.05 × NDF | 0.23 | Pulina and Rassu (1991) |
| 7 dairy breeds | > 1.5[a] | Indoors | MF = 1.89 + 0.012 × NDF | 0.48 | Serra (1998) |
| Comisana | > 1.5[a] | Grazing | MF = 15.157 − 0.022 × NDF | 0.36 | Cannas and Avondo (2002) |
| Dairy breeds | > 1[a] | Mostly indoors | MF = 1.68 + 0.014 × NDF | 0.43 | Pulina *et al.* (2006b) |

[a] Below this production level there was no association between NDF and milk fat.

© Woodhead Publishing Limited, 2010

short-chain FA in the udder, and decrease the availability of metabolites (mainly FA from lipoproteins) that the mammary gland takes up from the blood.

*Rumen-protected/inert fat supplements*

The most commonly used rumen-protected/inert fats are calcium soaps of fatty acids (CSFA), and the most used CSFA is the calcium soap of palm oil (CSPO). A review of experiments in which CSPO was used in lactating sheep demonstrated that addition of CSPO to the diet caused consistent increases in milk fat concentration and yield (Pulina *et al.* 2006a). In most cases, CSPO caused reductions in milk protein concentration and yield, and sometimes the reduction was quite strong (for example, −17%; Horton *et al.* 1992). However, protein yield was not always reduced. CSPO interacts with the non-structural carbohydrate (NSC) source to influence milk fat content and yield. The stage of lactation and duration of the experiments influenced strongly the variability of the results among the different experiments. The variability in the results among experiments may also be due to differences in feeding techniques (e.g. grazing vs. indoor feeding), in the amount of pasture eaten in grazing studies, and in the way the supplement was fed (e.g. mixed in the concentrate supplied at milking or added to a total mixed ration).

Inclusion of other inert rumen fat sources, such as the calcium soap of olive oil, induced effects on milk fat similar to those reported for the calcium soap of palm oil. In one study by Kitessa *et al.* (2003) supplementation of inert polyunsaturated FA from tuna oil did not affect milk yield, or its fat, protein and lactose composition, over a 10-day feeding period in different sheep breeds. Although the inclusion of dietary fat has been reported to induce a reduction in milk protein synthesis, very little information is available on its effects on milk protein composition in sheep (Goulas *et al.* 2003, Zhang *et al.* 2006).

*Rumen-unprotected fat supplements*

The addition of unprotected lipids to the diet of dairy ewes has produced contrasting results on the content of their milk fat (Table 13.5). For example, Zervas *et al.* (1998) observed that the addition of 50 g/kg soybean oil to the concentrate increased milk yield and decreased milk fat and protein concentrations. Similarly, Sarda ewes supplemented with soybean oil (38.5 g/kg of DM) at two forage to concentrate ratios (75:25 and 60:40), had a significant increase of milk and fat yield, a similar fat concentration, and a lower protein concentration compared to the non-supplemented control ewes (Mele *et al.* 2002). In Merino and Awassi ewes in mid-lactation, supplementation with complete pelleted diets containing 25 and 50 g/kg of sunflower oil had no effects on milk yield, or milk fat and protein content, and the clotting properties of the milk did not differ at any level of sunflower oil (Bencini 2005). Addition of ensiled crude olive cake (150 g/kg of DM for a mean addition of 12 g/kg of oil to the diet) did not influence milk yield, but increased milk fat content by 12% (Hadjipanayiotou 1999). The source of fat supplemented (e.g. flax and sunflower), and its physical form (seed or oil), may be a reason for differences in

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

© Woodhead Publishing Limited, 2010
IP Address: 129.132.208.100

**Table 13.5** Effects of free oil supplements on milk yield, milk fat content, fat yield, milk protein content and protein yield in dairy sheep. Differences are expressed in percentages with respect to control

| Lipid source | Dose (g/d) | Milk yield | Fat content | Fat yield | Protein content | Protein yield | Reference |
|---|---|---|---|---|---|---|---|
| Olive oil | 50 | +29.2 | −15.6 | +8.7 | −7.6 | +19.6 | Zervas *et al.* (1998) |
| Olive oil | 42 | +4.9 | +12.0 | +15.8 | nd | nd | Hadjipanayiotou (1999) |
| Olive oil | 100 | −4.8 | −22.9 | −25.7 | −14.0 | −21.7 | Porcu *et al.* (2003)[a] |
| Olive oil | 148 | +13.6 | −2.2 | +17.2 | −3.8 | +8.5 | Gómez-Cortés *et al.* (2008) |
| Soybean oil | 100 | +4.4 | +0.3 | +13.3 | −3.9 | +8.2 | Mele *et al.* (2002) |
| Sunflower oilseeds | 78 | −3.8 | −3.9 | −7.6 | −2.3 | −6.3 | Zhang *et al.* (2006) |
| Flax oilseeds | 68 | +8.4 | −2.2 | +6.1 | −1.1 | +7.2 | Zhang *et al.* (2006) |
| Tallow 0-21 DIM | 175 | +15.7 | −14.4 | −1.8 | −12.0 | +1.9 | Goulas *et al.* (2003) |
| Tallow 0-21 DIM | 137 | +6.4 | −13.1 | −8.8 | +3.4 | +10.1 | Goulas *et al.* (2003) |
| Tallow + meth. 0-21 DIM | 188 + 13 | +37.3 | −5.4 | +26.0 | −10.1 | +23.4 | Goulas *et al.* (2003) |
| Tallow + meth. 22-91 DIM | 155 + 11 | +34.4 | −12.4 | +10.6 | +0.8 | +35.3 | Goulas *et al.* (2003) |

[a] The values for Porcu *et al.* (2003) refer to the last two experimental days.

responses among different studies. The differences between the fat sources are likely to be related to their FA composition and/or their different seed size: sunflower seeds are much larger than flax seed, and this might have influenced rumination and rumen passage, thus affecting the availability of oil in the rumen (Zhang *et al.* 2006). Supplementation of lactating ewes with animal fats decreased milk fat concentration but did not change fat and protein yields (Goulas *et al.* 2003). When the same fat source was used with a source of rumen-protected methionine, there was a marked increase in milk yield, milk fat and milk protein yield in early lactation, but these effects were less pronounced in mid-lactation (Goulas *et al.* 2003).

*Milk fat depression syndrome in sheep*

A drastic reduction in the concentration of milk fat, also known as milk fat depression (MFD), occurs when dietary fibre is low and unprotected plant oils or fish oils are fed. MFD in dairy cows was positively associated with *trans*-10, *cis*-12 CLA concentration in milk fat (Bauman and Griinari 2003). Compared to dairy cows, MFD in dairy sheep is less common. No reduction in milk fat concentration was observed upon changing NDF level (240 vs 440 g/kg of DM; Nudda *et al.* 2004b) or the forage:concentrate ratio (60:40 vs. 75:25 supplemented, or not, with soybean oil; Mele *et al.* 2002); indeed, fat addition increased milk fat yield. Even the use of pelleted diets with very low fibre as the only feed did not induce MFD (Rossi *et al.* 1991). A reason why MFD is not common in ewes may be that they are able to ruminate and have normal rumen function when fed finely ground diets that cause rumen acidosis in cows (Cannas 2004). By contrast, in dairy sheep fed low fibre diets, supplementation with 100 g of free olive oil for one week did not modify milk yield, but it decreased milk fat and protein concentration and yield in the last two experimental days (Porcu *et al.* 2003). These different results may be due to an interaction between low fibre and high oil addition, which are the two conditions necessary to induce MFD (Bauman and Griinari 2003).

IP Address: 129.132.208.100

*Dietary protein*

Attempts to increase milk protein content through dietary crude protein (CP) supplementation have often resulted in no effects, or in small changes (Bocquier and Caja 2001). Diets with an increasing dietary CP concentration, at two NE levels, did not change the milk protein concentration but did increase milk protein yield (Cannas *et al.* 1998). However, protein concentration and daily protein yield increased in the milk of ewes in which the dietary CP concentration was initially very low (i.e., 100–110 g/kg DM) and was increased up to 140 g/kg DM (Calderon Cortes *et al.* 1977, Cowan *et al.* 1981). Increases in dietary protein generally are accompanied by increases in food intake. The consequent increase in milk production masks the increased synthesis of protein in the mammary gland, and the concentration of protein in the milk does not change (Robinson *et al.* 1974, Cowan *et al.* 1981, Penning *et al.* 1988, Frey *et al.* 1991, Hatfield *et al.* 1995).

© Woodhead Publishing Limited, 2010

Dietary protein sources can influence the yield and protein concentration of sheep milk as a result of the balance between the dietary essential amino acids and their requirement by dairy ewes. Fish meals have an amino acid composition that is very close to optimal for milk protein synthesis compared to other protein sources, such as soybean meal, linseed or peanut proteins. This explains the highest milk, and milk protein, yield in ewes fed fish meal (Purroy and Jaime 1995, Gonzales *et al.* 1982).

Pulina *et al.* (2006a) reviewed the effects of supplementation of methionine (Met) and lysine (Lys) on milk yield and protein content in ewes. In sheep, supplementation of ruminally protected Met and Lys was used to increase the efficiency of N utilization for milk protein synthesis (Lynch *et al.* 1991), and supplementation caused a slight increase of the Met and Lys content in milk, which was associated with a higher growth of lambs suckling this milk.

The protein content of the diet affects the quantity and the partition of nitrogenous substances in the milk. In general, an increase of dietary crude protein (CP) did not affect milk CP content, but influenced the milk N fraction. In sheep, there is a close positive relationship between milk urea (MU) and dietary CP concentration (Fig. 13.2, Cannas *et al.* 1998), probably because the efficiency of utilization of dietary N for milk protein synthesis decreased, and N losses increased, as CP content in the diets increased.

In addition, high-energy diets reduce the necessity for ewes to use amino acids as an energy source, so that less ammonia is produced from amino acid catabolism. Effects of dietary rumen-degradable organic matter and rumen-degradable CP on milk urea (MU) were examined by Landau *et al.* (2005), who found differences of about 5–6 mg/dl of MU, depending on the ratio of rumen-degradable CP to rumen-degradable organic matter, for similar dietary CP concentrations.

IP Address: 129.132.208.100

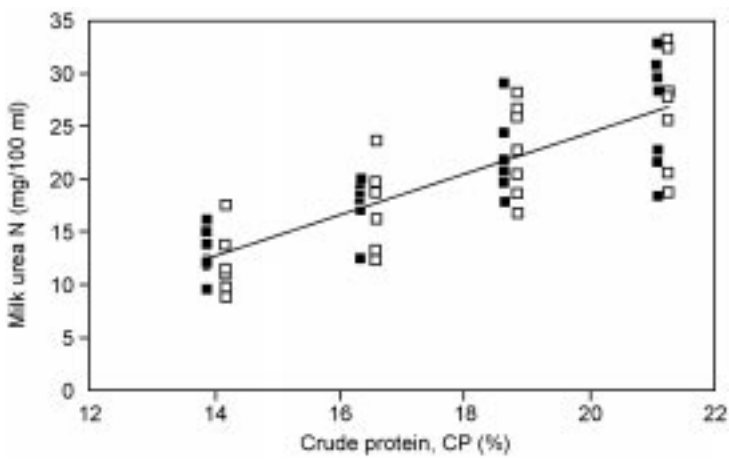



**Fig. 13.2** Relationship between the concentration of milk urea N and the percentage of dietary crude protein in ewes fed diets with high energy (□) and low energy (■) total mixed ratios (adapted from Cannas *et al.*, 1998).

© Woodhead Publishing Limited, 2010

*Alternative feed sources*

The use of alternative feeds (AF) in sheep nutrition has been reviewed by Vasta *et al.* (2008). Several researchers have shown that AF can be used successfully as supplements for sheep nutrition as a replacement for concentrates, without compromising animal performance. Some shrubs, legume seeds (e.g. *Pisum sativum*, *Cicer arietinum*, *Vicia faba*, *Ceratonia siliqua*), local agro-industrial by-products (e.g. olive cake, sugar beet pulp, extruded linseed cake and citrus pulp) or novel pasture species are widely available in Mediterranean countries and can reduce nutrition costs. Many of these alternative feeds contain secondary compounds, such as tannins. The effect of condensed tannins (CT) on milk composition depends on the CT concentration in the diet. When present in high concentrations in the diet, tannins can negatively affect animal productivity because they markedly reduce rumen microbial activity and bacterial and feed amino acid digestion in the intestine. In low amounts, tannins tend to increase milk yield and protein content, probably because they protect dietary proteins from ruminal degradation and increase the availability of essential amino acids. Moreover, the inclusion of alternative feeds in the diet can influence the appearance of nutraceutical or flavour-active compounds in milk and in dairy products. CT from *Hedysarum* (40 g/kg DM) offered to lactating sheep decreased the intestinal absorption of indole and skatole (Roy *et al.* 2004a) and their secretion in milk (Roy *et al.* 2004b). An increase in the concentration of polyunsaturated fatty acids (PUFA) in sheep milk was reported in dairy ewes grazing on *Hedysarum coronarium* compared to sheep fed on annual ryegrass, probably because the tannins in the *Hedysarum* reduced the biohydrogenation of PUFA in the rumen (Addis *et al.* 2005).

Vasta *et al.* (2008) also reviewed the use of alternative protein sources for dairy sheep. The partial or total replacement of soybean meal with chickpeas in the diets of sheep in mid-lactation did not affect milk yield and composition (Christodoulou *et al.* 2005). When soybean meal was substituted by isoproteic amounts of *Lupinus albus* in highly productive Sarda ewes in mid-lactation, no differences in milk yield, milk fat and protein content were observed between the two protein sources (Masucci *et al.* 2006). The use of agro-industrial by-products for sheep nutrition is an attractive proposition for sheep dairy farmers. Olive cake containing 115 g of fat/kg DM was used as silage to replace grass hay in the diet of Sarda ewes and did not modify milk yield or composition (Cabiddu *et al.* 2004).

The replacement of a starch-rich concentrate (500 g/d) with soybean hulls (350 g/d) and brewers grains (150 g/d) in the diet of dairy sheep increased milk yield and milk fat concentration but tended to decrease milk protein concentration (Cavani *et al.* 1990). Similarly, milk yield and milk fat concentration increased and milk protein concentration decreased when soybean hulls and beet pulps replaced corn and wheat grains in ewes in mid-lactation (Cannas *et al.* 1998). Zenou and Miron (2005) reported similar results on the effect of soybean hulls on milk yield and milk fat concentration in Assaf ewes. The replacement of grains, soybean meal, and wheat middlings with dried citrus pulps (300 g/kg of

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

concentrate DM) in the diet of low-productive dairy ewes did not affect milk yield or milk fat and protein concentrations but modified milk FA profile (Fegeros *et al.* 1995). The inclusion of increasing doses of citrus fruit (up to 30% on DM basis) in substitution of barley and beet pulp did not affect the milk composition or the biochemical and sensory characteristics of cheese (Jaramillo *et al.* 2009). By contrast, the partial replacement of an oat hay and concentrate mixture with silage made from oranges (400 g/kg of dietary DM) in the diet of dairy ewes decreased milk yield and milk protein concentration, probably due to a reduced microbial protein synthesis and flow to the intestine (Volanis *et al.* 2004). The supplementation of wet lemon (*Citrus limon* L.) pulp to Valle del Belice ewes grazing on natural pastures positively influenced milk yield and decreased milk protein content but did not modify milk fat concentration and milk coagulation properties (Todaro *et al.* 2004).

The use of novel forage and pasture species to increase pasture quality and availability in late spring and early summer has not been fully investigated in the Mediterranean areas. Grazing Sarda ewes on safflower, chicory and burr medic produced no differences in milk yield among the three species, while safflower and chicory-fed ewes produced milk with lower fat and protein concentrations than ewes fed on burr medic. The safflower diet caused a higher reduction of milk fat and protein concentration than the chicory diet, probably because of its higher content of total polyphenols and tannins compared to that of the other two species, which might have reduced rumen microbial activity (Landau *et al.* 2005). The above results highlight the fact that the use of alternative sources of protein is quite site specific and results can vary depending on many different factors, but especially on the quality of locally available food sources.

IP Address: 129.132.208.100

*Pasture*

Forage quality and its availability have significant effects on milk composition. The type of forage, its conservation and botanical composition all influence milk quality.

Pulina *et al.* (2005) re-elaborated the results of 43 groups of lactating Sarda sheep from 18 different experiments in animals fed exclusively on pasture (P), compared to grazing ewes supplemented by 0.5 kg/d of concentrate (P+C) and ewes fed exclusively a complete pelleted diet (C). Supplementing grazing with concentrates did not increase milk production when compared to grazing alone. Higher milk yield generally resulted in lower fat and protein concentrations, so that the differences in daily yield of total utilizable substances (TUS = the quantity of fat and protein produced daily by a single animal) were lower (Table 13.6). However, ewes fed a complete pelleted diet produced more TUS than grazing sheep ($P < 0.001$) because they had significantly higher intakes. This resulted in a favourable efficiency feeding index, but the feeding cost per kilogram of total utilizable substances (TUS) produced was much higher than in regimes which used only grazing. This result justifies the growing importance of pasture as a food source, given that intensive systems have lower labour costs

© Woodhead Publishing Limited, 2010

© Woodhead Publishing Limited, 2010
IP Address: 129.132.208.100

**Table 13.6** Food intake, milk yield and composition, efficiency feeding index and feeding costs of Sarda dairy ewes under three different feeding regimes

| Feeding | Number of trials, animals | Intake (g/d) | Milk yield (g/d) | Milk fat (%) | Milk protein (%) | Efficiency feeding index (DM/milk) (kg) | Cost of TUS[a] (euro/kg) |
|---|---|---|---|---|---|---|---|
| Complete pelleted diets | 12, 86 | 2497[b] | 1791[b] | 5.57[c] | 5.38[c] | 1.419 | 3.83[b] |
| Pasture + concentrates | 12, 744 | 1850[c] | 1251[c] | 6.48[b] | 5.84[c] | 1.677 | 2.04[c] |
| Pasture | 19, 1078 | 1704[c] | 1087[c] | 6.56[b] | 5.88[b] | 1.712 | 1.35[d] |
| *P* value | | <0.001 | <0.001 | 0.003 | 0.057 | n.s. | <0.001 |

[a] TUS = total utilizable substances = fat + protein yield in kg. Cost (euro/kg) of DM: pasture 0.1, concentrates 0.3.
[b], [c] and [d] indicate significant differences at the levels shown in the last line.

but higher feeding and structural expenses per head. A mixed feeding regimen seems to be a good compromise, thanks to the interesting economic conversion index and the productive level achievable in groups fed on grazing supplemented with concentrates. The concentrate/pasture substitution index, that is the reduction in the amount of grass DM voluntarily eaten by a sheep supplemented with 1 kg of concentrate DM, is on average 0.69. This explains the positive, even if not significant, effect on milk yield of using concentrate supplements rather than grazing alone.

Pasture can also affect the flavour, antioxidant properties and fatty acid profile of the milk and dairy products derived from it. For instance, the botanical composition of pastures influences the proportions and quantities of monoterpenes and sesquiterpenes in milk fat and, consequently, plays a role in determining cheese flavour (Viallon *et al.* 2000).

Addis *et al.* (2006) observed that the inclusion of *Chrysanthemum coronarium* in a grass–legume based pasture composed of *Lolium rigidum* and *Medicago polymorpha* markedly influenced the occurrence of terpenes (terpinolene, triciclene and 3,7-dimethyl-1,6-octadiene) in the volatile fraction of sheep milk and cheese, and that cheese obtained from the milk of sheep grazing a mixture of ryegrass, *Medicago polymorpha* and *Chrysanthemum coronarium* could be distinguished from that made with the milk of sheep grazing a mixture of ryegrass and *Medicago polymorpha*. Similarly Carbonell *et al.* (2002) found the highest concentrations of terpenes, other aromatic compounds (e.g. toluene, naphthalene and phenol) and free FA in the cheese made from sheep milk produced in spring.

Sheep on pasture produce a milk with a higher content in liposoluble vitamins (A, E and D), which are then efficiently transferred into the cheese made from the milk (Lucas *et al.* 2006). Fresh forages also influence the fatty acid profile of milk fat. In general, the milk from pasture-fed ewes has a higher content in PUFA because grass lipids contain a high proportion of PUFAs compared to dry diets (Clapham *et al.* 2005).

In dairy ewes that are grazing pasture, the concentrations of C18:2, C18:3, *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA in milk were higher in April than in May (Nudda *et al.* 2003), possibly due to the decrease in forage quality that normally occurs as Mediterranean pasture plants mature from the vegetative to the reproductive stage. In cheese-making factories, bulk milk, cheese and ricotta analysed from March to June also showed a decrease in *cis*-9, *trans*-11 CLA and in *trans*-11 C18:1 content with season (Fig. 13.3, Nudda *et al.* 2005).

Levels of PUFA and CLA in milk are also affected by the botanical composition of the pasture. Higher levels of milk C18:3, *trans*-11 C18:1 and CLA were obtained when diets were based on pure legumes or grass–legume mixtures compared with pure grass pastures (Cabiddu *et al.* 2005). In a study comparing four forage species, the content of *cis*-9, *trans*-11 CLA in milk of ewes consuming *Chrysanthemum coronarium* and *Medicago polymorpha* was higher than in milk from sheep fed *Lolium rigidum* and *Hedysarum coronarium* (Addis *et al.* 2005).

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

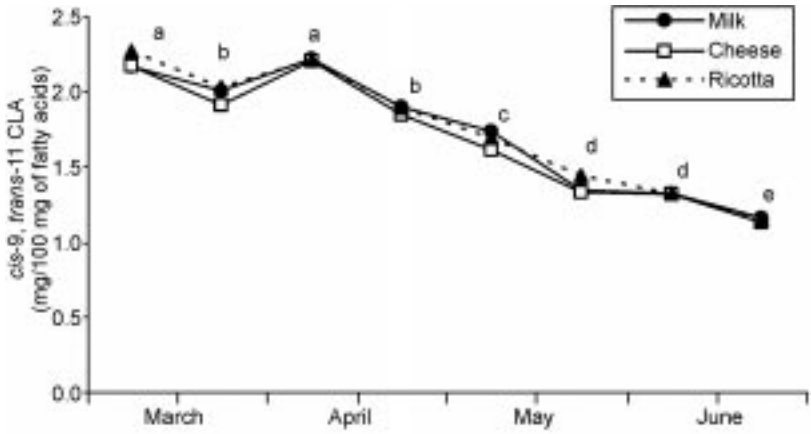



**Fig. 13.3** Seasonal evolution of *cis*-9, *trans*-11 CLA in sheep milk, cheese and ricotta sampled every two weeks from March to June in two milk processing plants in North Sardinia (adapted from Nudda *et al.*, 2005). a–e indicate significant ($P < 0.05$) differences between data points.

Designer milks with increased concentrations of C18:3 *n*-3 and CLA can be produced by supplementing the sheep diet with linseed oil, soybean oil or sunflower oil (Nudda *et al.* 2006, Mele *et al.* 2006, 2007, Bencini 2005).

IP Address: 129.132.208.100

### 13.6.4 Nutritional imbalances and SCC

The SCC in milk is indirectly affected by the nutrition of the ewe. For example, poor nutrition may predispose ewes to metabolic and infectious health disorders, which increase the susceptibility of the mammary gland to inflammation. Correct integration of vitamin A, or beta carotene, and vitamin E and Se in the ration maintains immune responses of mammary gland cells, thereby reducing the incidence of infections that can lead to increases in milk SCC (Morgante *et al.* 1999). Integration of the diet with these substances is particularly useful when ewes are fed mainly conserved fodders (i.e., hay or silage) that may have undergone considerable losses in beta-carotene and vitamin E during storage. Selenium deficiency can also compromise the antioxidant properties of the mammary tissue and cause an increase in the somatic cell count of the milk (Ronchi *et al.* 1994).

Improvement of the quality of the diet can reduce the somatic cell count in the milk, especially toward the end of lactation (Pulina *et al.* 1992). This is probably due to an improved functionality of the rumen, which results in the combined effect of increasing production, resulting in a dilution of the somatic cells and a slowing down of mammary cell turnover. Somatic cell counts can increase in sheep that are grazing green pasture due to excessive nitrogen intake (Cuccuru *et al.* 1994). The use of regulators of rumen fermentations such as Flavomycin (Hoechst Ltd) may result in reductions in the somatic cell count (Pulina and Rassu 1991).

© Woodhead Publishing Limited, 2010

Nutrition has a limited effect on the microbiological characteristics of sheep milk, which are mainly attributable to the hygienic conditions during milking. However, dietary imbalances (excess carbohydrates, excess nitrogen, insufficient fibre) causing anomalous fermentations in the rumen can provoke the output of highly contaminating faeces because of their low consistency and high content of microbial cells.

The use of silage can also increase the number of spore-forming bacteria, especially *Clostridium* spp., in the milk (Manfredini *et al.* 1987, Cavani *et al.* 1991), which could affect the keeping properties of the cheese.

## 13.7 Management of milking ewes

The milking operations and milking plants, the husbandry systems and housing environment and the health and hygienic conditions affect sheep milk quality in general, and the SCC and microbial cell content in particular. These factors are discussed below.

### 13.7.1 Milking operations

The milking operation in itself is critical to the production of high quality sheep milk. Even the transition between nursing their lambs and getting used to the milking parlour is a source of temporary stress for dairy ewes. The weaning of lambs and the start of machine milking in primiparous ewes that were exposed to this practice for the first time was found to be stressful, as shown by higher SCC in the milk for the first four days of machine milking (Rassu *et al.* 2006). For dairy sheep producers, a mixed system of suckling only during the day and once-daily machine milking during the morning for the first 30 days of lactation has been shown to be economically superior, in terms of lamb and milk production, both to the traditional system of lamb suckling with no machine milking during the first 30 days of lactation, and to a system where lambs were weaned at 24 h postpartum and the ewe was machine-milked twice daily (McKusick *et al.* 2001). The milk produced with the mixed weaning system is low in fat content and greater in SCC for the first 30 days of lactation (Gargouri *et al.* 1993, Fuertes *et al.* 1998, McKusick *et al.* 2002), which could be a disadvantage for cheese making (Requena *et al.* 1999). However, the mixed weaning system results in greater total lactation yields from the sheep and greater numbers of lambs weaned, which are both important in the economy of sheep dairy operations (Bencini 1999).

The success of the milking operations depends also on the behaviour and temperament of the sheep. Dimitrov-Ivanov and Djorbineva (2003) and Murray *et al.* (2006, 2009) found that calm ewes produced more milk than nervous ones. Sart *et al.* (2004) also reported that calm ewes produced milk that had a higher concentration of protein, suggesting that specific studies are needed to quantify the effect of temperament, behaviour and the interaction between people and dairy sheep.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

The concentration of protein and fat in the milk of ewes is no different with hand or machine milking. Hand milking elicited greater log SCC (6.07) than machine milking (5.94) in bulk milk. Machine milking of ewes in milking parlours (log SCC 5.88 to 5.94) was associated with better udder health than was the use of bucket-milking machines (6.04) in bulk milk samples (Gonzalo *et al.* 2005).

With mechanical milking, overmilking and excessively high vacuum levels, generally over 50 kPa at the milk line, can have a negative effect on teat condition (Peris *et al.* 2003) and increase the incidence of mastitis, because there is a positive correlation between the level of the vacuum and the SCC of milk (Fernandez *et al.* 1999). It is therefore paramount to avoid excessive levels of vacuum, vacuum instability and incorrect removal of the milking cups.

Lack of hygiene can result in contamination of the milk. Piovano *et al.* (2005) found that cleaning the udders, dipping after milking and the accurate washing of the milking machine reduced the microbial count of milk from 30 million/ml to 110,000/ml. By contrast, washing of the udders prior to milking is not recommended unless the udder is thoroughly dried, as dirty water dripping in the milking cups could otherwise contaminate the milk (Stubbs *et al.* 2009).

### 13.7.2 Seasonality and breeding out of season

As discussed previously, in the Mediterranean countries, sheep milking is seasonal, and sheep milk cheese is not produced during summer. In Europe when sheep milk is produced in summer the combined effects of seasonal changes in climate and herbage availability and variations in the metabolic status of the sheep have severe effects on the quality of the milk. The milk has long renneting times, poor consistency of the curd and high proteolytic and lipolytic activities, which result in poor processing performance (Delacroix-Buchet *et al.* 1994).

IP Address: 129.132.208.100

Jaeggi *et al.* (2005) processed sheep milk harvested in winter, spring and summer, and observed a decrease in total solids in the milk as the season progressed, corresponding to a decrease in the yield of cheese. While the effects of lactation and season are often confounded, this suggests that environmental factors may be important for the quality of sheep milk.

As discussed previously, the effects of photoperiod are controversial and while further work could be done to elucidate them, exposing animals to artificial light to increase milk production does not conform to the consumers' demand for clean, green and ethical animal products (Martin *et al.* 2004). For this reason it is sensible to adopt strategies to reduce the impact of high temperatures on lactating ewes in summer and provide an appropriate administration of mineral elements to sustain the yield and quality of milk and derived cheese (Sevi *et al.* 2004). In support of this, Pulina *et al.* (1993) have shown that a balanced ration restored the cheese-making performance of summer milk, suggesting that poor milk quality in summer may be attributed to the poor nutritive value of pasture.

© Woodhead Publishing Limited, 2010

Similarly, while sheep milk production through controlled reproduction and irrigated pastures in summer and early autumn was proposed to improve the efficiency of cheese factories (Piras *et al.* 2007), the problems with seasonal breeding mentioned above and the need to avoid the use of hormones make it possibly more viable to freeze the milk produced during the winter to maintain the supply of milk throughout the summer.

### 13.7.3 Mastitis

Subclinical infections caused by coagulase-negative staphylococci and other mammary pathogens in dairy ewes result in high SCC in the milk (Pengov 2001, Ariznabarreta *et al.* 2002), cause severe damage to the udder tissue (Burriel 1997) and result in significant losses of milk yield and milk components (Gonzalo *et al.* 2002). Although clinical mastitis decreases milk yield, subclinical mastitis in sheep is economically more important, because it is more frequent and is associated with a decrease in yield, quality and clotting properties of milk (Ruiu and Pulina 1992). On the other hand, the occurrence of subclinical mastitis may not be accompanied by the isolation of the etiological agent, probably because some enzymes may thwart pathogen detection.

The losses of milk yield through intramammary infection in sheep vary with the type of pathogen. Coagulase-negative staphylococci are the most prevalent pathogens in the mammary gland of sheep, and SCC is directly correlated to the losses in milk yield that result from these infections (Gonzalo *et al.* 2002). For this reason, SCC is the first and principal tool used by technicians and farmers to evaluate udder health in flocks. Breed, flock, stage of lactation, type of milking, installation within type of milking, dry therapy and contagious agalactia are important factors influencing SCC in dairy sheep (Gonzalo *et al.* 2005).

The upper threshold for SCC in a healthy ewe's udder should be 250,000 cells/ml (Pengov 2001). When a bulk tank SCC reaches levels above the upper threshold for good udder health (250,000 cells/ml), producers should begin to investigate possible udder health problems. High SCC also has a negative effect on milk processing, as milk with an SCC above 1,000,000 decreased the cheese yield and increased the development of rancid flavours in the cheese (Jaeggi *et al.* 2003).

Sarda breed ewes with mammary glands positive on bacteriological analysis suffered a reduction in total milk yield of about 24% during lactation when compared to negative animals (Fig. 13.4). The occurrence of intramammary infection before the peak of lactation caused a reduction in peak yield and, since milk yield loss was maintained during lactation, a consequent lower persistency was also observed (Pulina *et al.* 2007).

While direct selection for mastitis resistance has been considered inefficient because the heritability of SCC, as an indirect measurement of udder health, is low in dairy sheep (Lund *et al.* 1994), Bergonier and Berthelot (2003) proposed a method for estimating the presence of subclinical mastitis in ewes: in a series of checks of the same animal during lactation, an udder is considered 'healthy' if

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

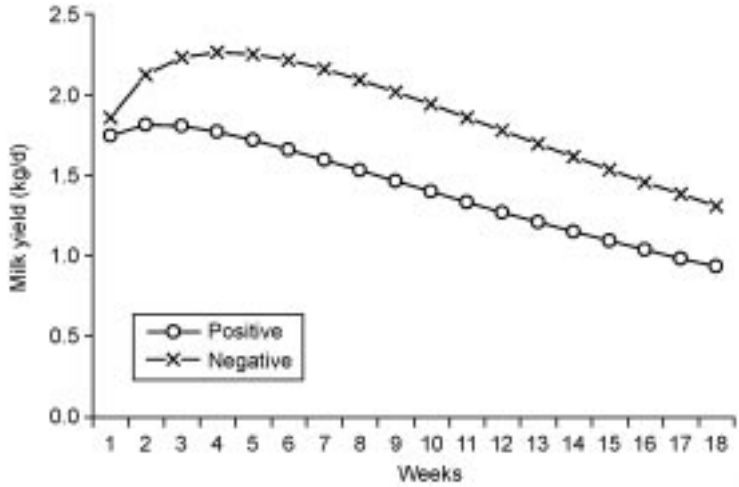



**Fig. 13.4** Lactation curves of dairy ewes positive and negative to the mastitis test (adapted from Pulina *et al.*, 2007).

every SCC (except possibly two) is below 500,000 cells/ml, 'infected' when at least two SCC are over 1 million cells/ml, and 'doubtful' between these two figures.

Implementing dry-ewe therapy significantly reduced log SCC to 5.91 compared with 6.10 when it was not implemented. As a result, dry therapy was proposed as the main tool to reduce SCC (Gonzalo *et al.* 2005). However, if we are to reduce the indiscriminate use of antibiotics it would be preferable to adopt the approach of Cuccuru *et al.* (2002), who demonstrated that simply by applying a correct milking routine for two years it is possible to reduce the bulk tank SCC from 2,124,000 cells/ml to 910,000 for machine milking and from 2,046,000 to 854,000 for hand milking.

IP Address: 129.132.208.100

### 13.7.4 Contaminants in sheep milk

A wide range of toxic undesirable substances, originating from anthropogenic and natural sources, could represent a potential source of risk to human health in the food chain. Mycotoxins, potentially toxic metals, such as heavy metals, and dioxins are the most relevant contaminants in feedstuffs and pastures used in feeding sheep. The extent of absorption of these undesirable substances from the gastrointestinal tract is one of the most important factors in determining the transfer of the contaminant into the milk of dairy sheep.

*Mycotoxins* are secondary metabolites produced by some species of moulds and are undesirable substances in foods and animal feeds. Mould growth and mycotoxin contamination of feedstuffs can occur at all stages of the productive cycle (i.e. cropping, harvest, transport, storage). Although some mycotoxins seem not to be toxic to sheep, at least at levels normally found in feeds, it is possible for some of their metabolites to be transferred to milk, creating a

© Woodhead Publishing Limited, 2010

potential risk to consumers. The most relevant mycotoxins occurring in feedstuffs are fusariotoxins, ochratoxin A and aflatoxins.

Fusariotoxins are mainly produced by some species of the *Fusarium* genus. The most frequently detected fusariotoxins in crops are trichothecenes (deoxynivalenol, nivalenol, T-2 and HT-2 toxins, etc.), zearalenone, and fumonisin B. For ruminants, ruminal metabolism is a determinant in milk contamination by fusariotoxins because the rumen microbial population has a strong detoxifying role (Yiannikouris and Jouany 2002). Studies on the presence of fusariotoxin residues in milk of ruminants indicate that, relative to the dose, only trace amounts were transferred into the milk. Therefore, even if fusariotoxins are toxic, they globally present a potential danger for animal and human health only when they are absorbed in great amounts or if exposure time is long (Cavret and Lecoeur 2006).

Ochratoxin A (OTA) is a mycotoxin produced by fungi of two genera: *Penicillium* and *Aspergillus*. OTA has been shown to be nephrotoxic, hepatotoxic, teratogenic and immunotoxic in several animal species (O'Brien and Dietrich 2005). In ruminants, the greater resistance to the toxic effects of OTA has been attributed to the capacity of the rumen microflora to hydrolyse the toxin yielding L-phenylalanine and ochratoxin-$\alpha$, which is non-toxic, or far less toxic than OTA (Hult *et al.* 1976). The disappearance of OTA from the rumen is faster for hay-fed than for grain-fed sheep (Xiao *et al.* 1991). However, conversion of OTA to ochratoxin-$\alpha$ is incomplete in sheep and leads to the appearance of intact OTA in blood and urine (Blank *et al.* 2003), resulting in a carryover of OTA of less than 1% in the milk of dairy sheep (Boudra *et al.* 2005).

Aflatoxins form a group of fungal toxins, produced mainly by *Aspergillus flavus* and *A. parasiticus*, which occur naturally in several important feedstuffs. Peanuts, maize grain and cottonseed are the major crops in which aflatoxins are produced. Aflatoxin B1 (AFB1) is the most toxic compound produced by these moulds. Aflatoxin M1 (AFM1) is the hydroxylated metabolite of AFB1 and may be transferred to the milk and milk products of animals that have ingested feeds contaminated by AFB1. The International Agency for Research on Cancer of WHO (IARC 2002) includes aflatoxins among the substances that are carcinogenic to humans (Group 1). Several countries have regulated the maximum permissible levels of AFB1 in food and AFM1 in milk and dairy products. The European Union fixed the limit for AFB1 at 5 $\mu$g/kg for dairy animals (European Commission 2003). The maximum allowed concentration of AFM1 in liquid milk for the European Union is fixed at 50 ng/kg (European Commission 2006). Several authors reported AFM1 contamination in samples of raw sheep milk (Roussi *et al.* 2002, Kaniou-Grigoriadou *et al.* 2005, Ghanem and Orfi 2009).

*In vitro* and *in vivo* studies of bovine and caprine rumen fluid suggested that ruminants could detoxify only part of the aflatoxins ingested in their digestive tract, mainly through the action of ruminal protozoa (Upadhaya *et al.* 2009).

The absorption of aflatoxins occurs in the rumen and the small intestine. The AFB1 is readily transported, probably by plasma lipoproteins, in the blood

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

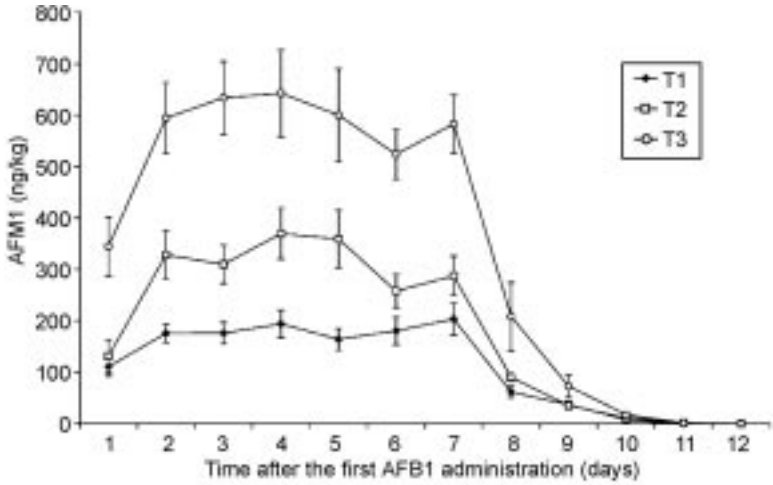



**Fig. 13.5** Aflatoxin M1 (AFM1) concentrations (mean ± SE) in the milk of ewes fed 32 (T1), 64 (T2) and 128 (T3) $\mu$g of AFB1 per day for 7 days (adapted from Battacone *et al.*, 2005).

vascular system (Wilson *et al.* 1985). When sheep were fed AFB1, the AFM1 appeared in the milk of the following milking (Battacone *et al.* 2003). The concentration of AFM1 in milk was dose dependent, and approached a steady state 2–3 days after the start of administration in diets contaminated with AFB1 artificially (Battacone *et al.* 2005; Fig. 13.5) or naturally (Battacone *et al.* 2009). However, the carry over of AFM1 in milk was markedly higher for naturally contaminated diet (range: 1.3 to 2.9%) than in the pure AFB1 administration (range: 0.26 to 0.33%). Because the AFM1 binds to ovine milk proteins, and in particular to the casein fraction (Barbiroli *et al.* 2007), it is concentrated in the curd during the manufacture of cheese (Battacone *et al.* 2005).

The contamination of pastures and the accumulation of potentially toxic *metals* in grazing sheep can occur on soils that are naturally rich in metals, following accidental or anthropogenic events, such as the fallout of radioactive contaminants on grassland, or prolonged use of sewage sludge used in agricultural practices. Because the systemic uptake of heavy metals by plants is limited, the main exposure route of grazing animals is probably through the ingestion of soil and soil-contaminated herbage (Abrahams and Steigmajer 2003).

Several authors have reported the presence of heavy metals in feeds, milk and sheep dairy products (Coni *et al.* 1999, Caggiano *et al.* 2005, Anastasio *et al.* 2006). Transfer of trace heavy metals into milk is a result of complex biomechanisms that, in many cases, require the activity of carrier proteins.

Transfer of cadmium (Cd) from feed to ovine milk was detected at the first milking (i.e. 6 h) after administration (Houpert *et al.* 1997). The milk Cd concentrations of ewes increased rapidly and the maximum level was on day 49 in an experiment in which ewes were fed 2 mg/kg/day of CdCl by granulated feed supplementation for 70 days. The concentration of Cd in rennet curds made with

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

the sheep milk was about five- to six-fold higher than that of whole milk, while the Cd concentration in lactic curds was about three times that in milk (Mehennaoui *et al.* 1999).

Even though Kan and Meijer (2007) reported a clear dose-dependent increase in heavy metal concentrations in the kidney and liver after dietary exposure, they concluded that high levels of heavy metals are not likely to be found in milk when animals are exposed via the diet.

*Dioxins* represent a group of toxic chemicals that may contaminate feedstuffs. Because they are highly persistent in the environment the contamination of feeds by dioxins will continue to occur in the future. Therefore efforts in the feed industry are needed to minimize the contamination of feedstuffs with dioxins. The family of dioxins includes polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs). These polychlorinated compounds are included in the category of endocrine disrupting compounds (EDCs) due to their ability to mimic, antagonize or disrupt the synthesis and metabolism of endogenous hormones and hormone receptors.

During the last 30 years the effects of dioxin exposure on animal cells have been the subject of intensive investigations in laboratory animals. Dioxins are bio-accumulative and have a common spectrum of biological responses mediated via binding to specific high-affinity cellular proteins. All dioxins enter the food chain through animals that are exposed to contaminated surroundings and feed, and tend to concentrate mainly in the body and milk fat. Dioxin levels in milk are related to emission from nearby industries (Schmid *et al.* 2003). Among the foods of animal origin, dairy products have the highest level of contamination (Bocio *et al.* 2003, Schecter *et al.* 2004). In a study on transfer of PCBs from blood into sheep milk or faeces, Vrecl *et al.* (2005) observed that the pattern of excreted PCB differed between two pathways. An enrichment of lipophilic precursors was observed in milk, because of its higher lipid content, compared with faeces. The distribution and clearance of many halogenated hydrocarbons have been studied in ruminants by administering known quantities of these compounds (Willett *et al.* 1993). Many of these hydrocarbons, including the precursors of PCB, have similar kinetic behaviour in lactating dairy cows and sheep (Willett *et al.* 1993, Busbee and Ziprin 1994, Vrecl *et al.* 2005). These studies suggest that in lactating sheep the higher chlorinated, coplanar and metabolically stable PCB precursors are preferentially excreted in milk and, due to their high toxicity, represent a potential risk to consumers. Because dioxins are highly lipophilic their excretion in milk is higher during the initial stages of lactation in which lipid mobilization is more intensive (Schulz *et al.* 2005).

In two sheep flocks grazing on grass contaminated with high dioxin levels, Perucatti *et al.* (2006) observed higher dioxin values in the milk than those observed in a control flock. The presence of dioxins as an environmental contaminant (i.e. soil, water and pasture) was related to an increase of the percentages of abortions, abnormal foetuses and chromosome abnormalities in the sheep grazed on the contaminated grass (Iannuzzi *et al.* 2004, Perucatti *et al.* 2006).

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

The application of sewage sludge to pastures could be the cause of the contamination of soil by EDCs other than dioxins, such as phthalate and alkyl phenol. In a study on grazing ewes, Rhind *et al.* (2007) reported that the concentrations of alkyl phenols in milk were low, and little higher than environmental concentrations, while phthalate concentrations were approximately double in milk than in the environment.

Pesticide residues in feed, as a consequence of their use in the production and storage of crops, could represent a potential risk if transferred in the milk of dairy ewes. Compounds with high lipid solubility tend to concentrate in tissues with higher fat content, such as adipose tissue and, in the case of lactating animals, milk. The transfer of lipid-soluble pesticides from contaminated feed to milk in lactating ruminants can be predicted using the mathematical model developed by MacLachlan and Bhula (2009) which takes into account the stage of lactation, as the volume of milk produced varies with days in milk and with the amount of body fat.

## 13.8 Developments in processing techniques for sheep milk

Sheep milk processing is one of the most ancient industries in the world: the first mention of it is in Homer's *Odyssey*, written in 700 BC!

In some countries, there is a perceived need to protect traditional techniques that identify DOC/DOP products (e.g. the famous Roquefort and Pecorino Romano). This has been supported by appropriate legislation in the countries of origin (Anifantakis 1990). Recent consumer trends such as the 'Slow Food' movement tend to encourage the retention of traditional techniques and also often long and complex maturation processes (Jones *et al.* 2003).

In countries with an emerging sheep milking industry, these products have often been 'copied' in an attempt to replace importations. However, the imitation of these traditional products has been discouraged and attempts have been made to develop novel products including cheeses made using vegetable rennets, mould-ripened cheeses, yoghurt and even ice-cream (Bencini 1999, Bencini and Agboola 2003, Stubbs *et al.* 2009). In these countries the production of dairy products requires that all milk is pasteurized before processing; this differs sharply from the Mediterranean countries where it is mandatory that some DOC cheeses be made from raw milk.

Research has established that sheep milk can be frozen without affecting its processing performance or the quality of the products (Bencini 1999, Katsiari *et al.* 2002). Apart from the obvious need of taking holidays, if all sheep could be dried up on a dairy farm, even for a short period, this would allow a period of rest for the milking paddocks (for instance, allowing a reduction of worm burdens) as well as time to carry out maintenance work at the dairy. The possibility of processing frozen milk could also save considerable sums of money currently spent on breeding sheep out of season.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

## 13.9 Sources of further information and advice

Apart from the authors, advice on sheep milking can be obtained from local branches of the Departments of Agriculture or similar organizations.

## 13.10 References and further reading

ABRAHAMS PW and STEIGMAJER J (2003) Soil ingestion by sheep grazing the metal enriched floodplain soils of mid-Wales, *Environmental Geochemical Health* 25, 17–24.

ADDIS M, CABIDDU A, PINNA G, DECANDIA M, PIREDDA G, PIRISI A and MOLLE G (2005) Milk and cheese fatty acid composition in sheep fed Mediterranean forages with reference to conjugated linoleic acid *cis*-9, *trans*-11, *Journal of Dairy Science* 88, 3443–3454.

ADDIS M, PINNA G, MOLLE G, FIORI M, SPADA S, DECANDIA M, SCINTU MF, PIREDDA G and PIRISI A (2006) The inclusion of a dairy plant (*Chrysanthemum coronarium*) in dairy sheep diet, 2 Effect on the volatile fraction of milk and cheese, *Livestock Production Science* 101, 68–80.

ALAIS C (1984) *Science du Lait. Principes des Techniques Laitières*, 4th edn, Sepaic, Paris.

ALEANDRI R, BUTTAZZONI LG and SCHNEIDER JC (1990) The effects of milk protein polymorphism on milk components and cheese-producing ability, *Journal of Dairy Science* 73, 241–255.

ANASTASIO A, CAGGIANO R, MACCHIATO M, PAOLO C, RAGOSTA M, PAINO S and CORTESI ML (2006) Heavy metal concentrations in dairy products from sheep milk collected in two regions of southern Italy, *Acta Veterinaria Scandinavica* 47, 69–73.

ANIFANTAKIS EM (1986) Comparison of the physico-chemical properties of ewe's and cow's milk. *Proceedings of the International Dairy Federation Seminar on Production and Utilization of Ewe's and Goat's Milk*, Athens, Greece, 23–25 September 1985. *Bulletin of the International Dairy Federation*, no. 202, 42–53.

ANIFANTAKIS EM (1990) Manufacture of sheep's milk products. *Proceedings of the XXIII International Dairy Congress*, Montreal, Quebec B 412–419.

ARA (1995) Laboratory of the Associazione Regionale Allevatori (Regional Breeders Association), Oristano, Sardinia, Italy.

ARAVE CW, GILLET TA, PRICE D and MATTHEWS DH (1973) Polymorphism in caseins of sheep milk, *Journal of Animal Science* 36, 241–244.

ARIZNABARRETA A, PONZALO C and SAN PRIMITIVO F (2002) Microbiological quality and somatic cell count of ewe milk with special reference to staphylococci, *Journal of Dairy Science* 85, 1370–1375.

ASKAR AA, HELAL FR, AHMED NS, HOFI AA and HAGGAG S (1984) Effect of seasonal variation on physical properties, gross composition, nitrogen distribution, rennin coagulation time and heat stability of Egyptian ewe's milk, *Egyptian Journal of Food Science* 12, 143–148.

BALTADJIEVA M, VEINOGLOU B, KANDARAKIS J, EDGARYAN M and STAMENOVA V (1982) La composition du lait des brebis de la région de Plovdiv en Bulgarie et de Ioannina en Grèce [The composition of milk form sheep in the regions of Plovdiv in Bulgaria and Ioannina in Greece], *Lait* 62, 191–201.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

BARBIROLI A, BONOMI F, BENEDETTI S, MANNINO S, MONTI L, CATTANEO T and IAMETTI S (2007) Binding of aflatoxin M1 to different protein fractions in ovine and caprine milk, *Journal of Dairy Science* 90, 532–540.

BARILLET F (1997) Genetics in milk production, in L Piper and A Ruwinsky, *The Genetics of Sheep*, CAB International, London, 539–564.

BARILLET F, ELSEN JM and ROUSSELY M (1986) Optimization of a selection scheme for milk composition and yield in milking ewes: example of the Lacaune breed, *Proceedings of the 3rd World Congress on Genetics Applied to Livestock Production* 6, 658–664.

BARNICOAT CR (1952) Milk production of the ewe, *Proceedings of the New Zealand Society for Animal Production* 12, 115–120.

BATTACONE G, NUDDA A, CANNAS A, CAPPIO BORLINO A, BOMBOI G and PULINA G (2003) Excretion of aflatoxin M1 in milk of dairy ewes treated with different doses of aflatoxin B1, *Journal of Dairy Science* 86, 2667–2675.

BATTACONE G, NUDDA A, PALOMBA M, PASCALE M, NICOLUSSI P and PULINA G (2005) Transfer of aflatoxin B1 from feed to milk and from milk to curd and whey in dairy sheep fed artificially contaminated concentrates, *Journal of Dairy Science* 88, 3063–3069.

BATTACONE G, NUDDA A, PALOMBA M, MAZZETTE A and PULINA G (2009) The transfer of aflatoxin M1 in milk of ewes fed diet naturally contaminated by aflatoxins and effect of inclusion of dried yeast culture in the diet, *Journal of Dairy Science*, in press.

BATTAGLINI A and DE MARIA C (1977) Influenza della soppressione di una mungitura giornaliera sulla produzione e su talune caratteristiche chimico fisiche del latte di pecore di razza Sopravissana [Influence of omitting one daily milking on milk production and on some chemical and physical characteristics of the milk of Sopravissana ewes], *Annali dell'Istituto Sperimentale per la Zootecnia* 10, 73–92.

BATTAGLINI A, DE MARIA C, DELL'AQUILA S and TAIBI L (1977) Influenza della soppressione di una mungitura giornaliera sulla produzione e su talune caratteristiche chimico-fisiche del latte prodotto da un gruppo di pecore Wurttemberg × (Ile de France × Gentile di Puglia) [Influence of omitting one daily milking on milk yield and on some chemical and physical characteristics of the milk produced by a group of Wurttemberg × (Ile de France × Gentile di Puglia) ewes], *Annali dell'Istituto Sperimentale per la Zootecnia* 10, 123–135.

BATTAGLINI A, DE MARIA C, DELL'AQUILA S and TAIBI L (1979) Effetti della soppressione di una mungitura giornaliera sulla produzione e su talune caratteristiche qualitative del latte di pecore di razza Comisana [Effects of omitting one daily milking on milk production and on some qualitative characteristics of the milk of Comisana ewes], *Annali dell'Istituto Sperimentale per la Zootecnia* 12, 1–11.

BAUMAN DE (1987) Somatotropin and lactation, *Journal of Dairy Science* 70, 474–86.

BAUMAN DE (1999) Bovine somatotropin and lactation: from basic science to commercial application, *Domestic Animal Endocrinology*, special issue 17, 101–116.

BAUMAN DE and GRIINARI JM (2003) Nutritional regulation of milk fat synthesis, *Annual Review of Nutrition* 23, 203–227.

BENCINI R (1993) The sheep as a dairy animal: lactation, production of milk and its suitability for cheese making. PhD thesis, The University of Western Australia.

BENCINI R (1999) *Development of specialty sheep milk dairy products – Increasing the market scope*. Rural Industries Research and Development Corporation Publ. No. 99/69.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

BENCINI R (2002) Factors affecting the clotting properties of sheep milk, *Journal of the Science of Food and Agriculture* 85, 705–719.

BENCINI R (2005) *Dairy products and farming techniques for the sheep milking industry*. Rural Industries Research and Development Corporation Publ No. 05/142.

BENCINI R and AGBOOLA SO (2003) *Marketable products from sheep milk*. Rural Industries Research and Development Corporation Publ. No. 02/143.

BENCINI R and JOHNSTON K (1996) Factors affecting the clotting properties of sheep milk. *International Dairy Federation Bulletin*, special issue on production and utilization of sheep and goats milk 3, 199–204.

BENCINI R and KNIGHT TW (1994) Double cupping and machine stripping optimise the yield and the composition of sheep milk, *Proceedings of the Australian Society of Animal Production* 20, 171–174.

BENCINI R and PULINA G (1997) The quality of sheep milk. A review, *Australian Journal of Experimental Agriculture* 37, 485–504.

BENCINI R and PURVIS IW (1990) The yield and composition of milk from Merino sheep, *Proceedings of the Australian Society of Animal Production* 18, 144–148.

BENCINI R, KNIGHT TW and HARTMANN PE (2003) Secretion of milk and milk components in sheep, *Australian Journal of Experimental Agriculture* 43, 529–534.

BERGONIER D and BERTHELOT X (2003) New advances in epizootiology and control of ewe mastitis, *Livestock Production Science* 79, 1–16.

BLANK R, ROLFS JP, SUDEKUM KH, FROHLICH AA, MARQUARDT RR and WOLFFRAM S (2003) Effects of chronic ingestion of ochratoxin A on blood levels and excretion of the mycotoxin in sheep, *Journal of Agricultural and Food Chemistry* 51, 6899–6905.

BOCIO A, LLOBET JM, DOMINGO JL, CORBELLA J, TEIXIDO A and CASAS C (2003) Polybrominated diphenyl ethers (PBDEs) in foodstuffs: human exposure through the diet, *Journal of Agricultural and Food Chemistry* 5, 3191–3195.

BOCQUIER F and CAJA G (1993) Recent advances on nutrition and feeding of dairy sheep, *Proceedings of the 5th International Symposium on Machine Milking of Small Ruminants*, *Hungarian Journal of Animal Production* Suppl. 1, 580–607.

BOCQUIER F and CAJA G (2001) Production et composition du lait de brebis: effets de l'alimentation [Production and composition of milk from sheep: effects of nutrition], *Production Animal* 14(2), 129–140.

BOCQUIER F, KANN G and THERIEZ M (1990) Relationships between secretory patterns of growth hormone, prolactin and body reserves and milk yield in dairy ewes under different photoperiod and feeding conditions, *Animal Production* 51, 115–125.

BOLLA P, CAROLI A, RIZZI R and ACCIAIOLI A (1986) Relazioni tra polimorfismo della $\beta$-lattoglobulina e caratteri produttivi della pecora Massese [Relationships between $\beta$-lactoglobulin polymorphism and productive traits in Massese sheep], *Proceedings of the SISVET (Italian Society of Veterinary Science)* 40, 591–595.

BOLLA P, CEROTTI G and CAROLI A (1985) Analisi delle proteine del latte ovino mediante isoelettrofocalizzazione [Analysis of sheep milk proteins by isoelectric focusing], *Proceedings of the SISVET (Italian Society of Veterinary Science)* 39, 399–402.

BOUDRA H, ALVAREZ D, JOUANY J-P and MORGAVI DP (2005) Transmission of ochratoxin A into ewe's milk following a single or chronic ingestion of contaminated feed, *Proceedings of the World Mycotoxin Forum, Third Conference*, Noordwijk, The Netherlands, Bastiaanse Communications, Bilthoven, The Netherlands.

BOYAZOGLU J and MORAND-FEHR P (2001) Mediterranean dairy sheep and goat products and their quality. A critical review, *Small Ruminant Research* 40, 1–11.

BOYAZOGLU JG (1963) Aspect quantitatifs de la production laitière des brebis [Quantita-

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

tive aspects of milk production in sheep], *Annales de Zootechnie* 12, 237–296.

BURRIEL AR (1997) Dynamics of intramammary infection in the sheep caused by coagulase-negative staphylococci and its influence on udder tissue and milk composition, *Veterinary Record* 140, 419–423.

BURRIS J and BAUGUS CA (1955) Milk consumption and growth of suckling lambs, *Journal of Animal Science* 14, 186–191.

BUSBEE DL and ZIPRIN RL (1994) Gastrointestinal uptake and vascular transport of 2,4'-dichlorobiphenyl, *Archives of Toxicology* 68, 96–102.

CABIDDU A, CANU M, DECANDIA M, MOLLE G and POMPEL R (2004) The intake and performance of dairy ewes fed with different levels of olive cake silage in late pregnancy and suckling periods, in H Ben Salem, A Nefzaoui and P Morand-Fehr, eds, *Nutrition and Feeding Strategies of Sheep and Goats under Harsh Climates*, CIHEAM-IAMZ, *Options Méditerranéennes*, Série A, Zaragoza, Spain, 197–201.

CABIDDU A, DECANDIA M, ADDIS M, PIREDDA G, PIRISI A and MOLLE G (2005) Managing Mediterranean pastures in order to enhance the level of beneficial fatty acids in sheep milk, *Small Ruminant Research* 59, 169–180.

CAGGIANO R, SABIA S, D'EMILIO M, MACCHIATO M, ANASTASIO A, RAGOSTA M and PAINO S (2005) Metal levels in fodder, milk, dairy products, and tissues sampled in ovine farms of Southern Italy, *Environmental Research* 99, 48–57.

CALDERON-CORTES JF, ROBINSON JJ, MCHATTIE I and FRASER C (1977) The sensitivity of ewe milk yield to changes in dietary crude protein concentration, *Animal Production* 24, 135.

CANNAS A (2004) Feeding of lactating ewes, in G Pulina and R Bencini, *Dairy Sheep Nutrition*, CABI Publishing, Wallingford, 79–108.

CANNAS A and AVONDO M (2002) Relationships among milk fat content, energy balance and NDF intake in lactating ewes fed at pasture, *Proceedings of the 53rd Annual Meeting of EAAP – European Association of Animal Production*, Cairo, 55.

CANNAS A, PULINA G, RASSU SPG and MACCIOTTA NPP (1991) Influenza della terza mungitura sulla produzione quanti-qualitativa in pecore di razza Sarda [Influence of a third milking on quantity and quality of milk produced by Sarda sheep], *Proceedings of the SISVET (Italian Society of Veterinary Science)* 45, 1769–1772.

CANNAS A, PES A, MANCUSO R, VODRET B and NUDDA A (1998) Effect of dietary energy and protein concentration on the concentration of milk urea nitrogen in dairy ewes, *Journal of Dairy Science* 81, 499–508.

CAPPIO-BORLINO A, MACCIOTTA NPP, PULINA G, ROSATI A and ROSSI G (1996) Geni maggiori e QTL, in CNR-RAISA, *Ricerche e Applicazioni in Zootecnia*, AP Publications, Rome.

CARBONELL M, NUNEZ M and FERNANDEZ-GARCIA E (2002) Seasonal variation of volatile compounds in ewe raw milk La Serena cheese, *Lait* 82, 699–711.

CAROLI A, BOLLA P, PAGNACCO G and FRAGHI A (1989) Studio sul controllo genetico del fenotipo Welsh di $\alpha_s$-caseina nella pecora [Study on genetic control of the Welsh phenotype for $\alpha_s$-casein in the sheep], *Proceedings of the 24th Simposio Internazionale di Zootecnia* 275–282.

CARTA A and ELSEN JM (1999) Sire power design calculation for QTL mapping experiments, *Genetics Selection Evolution* 31, 183–189.

CARTA A, PIREDDA G, ADDIS M, CABIDDU A, FIORI M, LEROUX C and BARILLET F (2003) Fatty acid composition of sheep milk from a back-cross Sardinian × Lacaune resource population: preliminary QTL detection for CLA content, *Options Méditerranéennes* Série A 55, 107–113.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

CASOLI C, DURANTI E, MORBIDINI L, PANELLA F and VIZIOLI V (1989) Quantitative and compositional variations of Massese sheep milk by parity and stage of lactation, *Small Ruminant Research* 2, 47–62.

CASOLI C, DURANTI E and MAHRABI H (1990) Valutazione dell'attitudine alla coagulazione del latte ovino [Evaluation of the aptitude for coagulation of sheep milk], *Proceedings of the SISVET (Italian Society of Veterinary Science)* 44, 1671–1675.

CASOLI C, PAUSELLI M, MORGANTE M, RANUCCI S, DURANTI E and MEHRABI H (1992) Comportamento reologico del latte ovino in rapporto alle caratteristiche chimico-fisiche e cellulari [Rheological behaviour of sheep milk in relation to its chemical, physical and cellular characteristics], *Proceedings of the 10th SIPAOC Congress* 250–251.

CASU S and BOYAZOGLU JG (1974) Effets de la suppression de la traite du soir chez la brebis Sarde [Effects of the suppression of the evening milking in the Sarda sheep], *Proceedings of the Symposium sur la Traite Mécanique des Petits Ruminants*, Millau, France, 7–11 May 1973. *Annales de Zootechnie*, Numéro Hors Série/1974 139–144.

CASU S and LABUSSIÈRE J (1972) Premiers résultats concernant la suppression d'une ou plusieurs traites par semaine chez la brebis Sarde [First results on the suppression of one or more milkings per week in the Sarda sheep], *Annales de Zootechnie* 21, 223–232.

CASU S and SANNA S (1990) Aspetti e problemi del miglioramento genetico della composizione del latte di pecora e di capra [Aspects and problems of genetic improvement of the composition of milk from sheep and goats], *Proceedings of the 2nd International Symposium 'Nuove Prospettive della Ricerca sugli Ovi-caprini'*, Varese, Italy, 171–195.

CASU S, BOYAZOGLU JG and RUDA G (1978a) Essais sur la traite mechanique semplifiee des brebis Frisonne × Sarde [Tests on simplified mechanical milking of East Friesian × Sarda sheep], *Proceedings of the Symposium sur la Traite Mécanique des Petits Ruminants*, Alghero, Italy, 235–243.

CASU S, SANNA A, SANNA G and PICCINELLI G (1978b) Simplification des operations de la traite mécanique des brebis Sardes: la suppression de la repasse à la machine [Simplification of mechanical milking operations in Sarda sheep: suppression of mechanical stripping], *Proceedings of the Symposium sur la Traite Mécanique des Petits Ruminants*, Alghero, Italy, 215–223.

CASU S, FRESI P and CARTA A (2002) Estimation of the genetic parameters of udder traits in Sarda dairy sheep using a sire model with random contemporary groups, *Proceedings of the 7th World Congress on Genetics Applied to Livestock Production*, Session 09 Lactation and milk quality, Communication No. 04, Montpellier, France.

CASU S, CARTA A and ELSEN JM (2003) Strategies to optimize QTL detection designs in dairy sheep populations: the case of Sarda breed, *Options Méditerranéennes* 55, 19–23.

CASU S, COLOMBINO M, MULAS G, SECHI S, BARILLET F and CARTA A (2009) Detection on OAR7 of QTL affecting fat and protein yields in dairy sheep, *Italian Journal of Animal Science* 8, suppl. 2, 45–47.

CAVANI C, BIANCONI L and MONGARDI D (1990) Soybean hulls and cereal distillers in dairy sheep feeding, *Proceedings of the 9th SIPAOC Congress*, Grado, Italy, 6.09–6.10.

CAVANI C, BIANCONI L, MANFREDINI M, RIZZI L and ZARRI MC (1991) Effects of a complete diet on the qualitative characteristics of ewe milk and cheese, *Small Ruminant Research* 5, 273–284.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

CAVRET S and LECOEUR S (2006) Fusariotoxin transfer in animal, *Food Chemical Toxicology* 44, 444–453.

CHILLIARD Y and BOCQUIER F (2000) Direct effects of photoperiod on lipid metabolism, leptin synthesis and milk secretion in adult sheep, in P Cronjé and EA Boomkerand, *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*, CAB International, London.

CHIOFALO L and MICARI P (1987) Attuali conoscenze sulle varianti delle proteine del latte nelle popolazioni ovine allevate in Sicilia. Osservazioni sperimentali [Current knowledge on the variants of milk proteins in ovine populations farmed in Sicily. Experimental observations], *Scienza e Tecnica Lattiero Casearia* 38, 104–114.

CHIOFALO L, MICARI P and STURNIOLO G (1982) Polimorfismo delle proteine del latte nella pecora Siciliana [Polymorphism in the proteins of milk in Sicilian sheep], *Zootecnia e Nutrizione Animale* 8, 263–268.

CHIOFALO L, MICARI P, CHIOFALO V and GIUNTA P (1989) Parametri chimici e di coagulazione nel latte delle razze Sarda e Comisana [Chemical and coagulation parameters in the milk of the Sarda and Comisana breeds], *Proceedings of the SISVET (Italian Society of Veterinary Science)* 43, 1851–1856.

CHRISTODOULOU V, BAMPIDIS VA, HUČKOB B, PLOUMI K, ILIADIS C, ROBINSON PH and MUDŘIKB Z (2005) Nutritional value of chickpeas in rations of lactating ewes and growing lambs, *Animal Feed Science and Technology* 118, 229–241.

CLAPHAM WM, FOSTER JG, NEEL JPS and FEDDERS JM (2005) Fatty acid composition of traditional and novel forages, *Journal of Agricultural and Food Chemistry* 53, 10068–10073.

CLÉMENT P, AGBOOLA SO and BENCINI R (2006) A study of polymorphism in milk proteins from local and imported breeds in Australia by capillary electrophoresis, *Lebensmittel-Wissenschaft und Technologie* 39, 63–69.

CLUTTON-BROCK J (1981) *Domesticated Animals from Early Times*, British Museum (Natural History), London.

COGAN TM and HILL C (1993) Cheese starter cultures, in PF Fox, *Cheese: Chemistry, Physics and Microbiology*, Chapman & Hall, London, vol. 1, 193–255.

COLUMELLA LJM (*circa* 4 BC to 65 AD) On the purchase and care of sheep, in *De Re Rustica*, William Heinemann Ltd, London and Harvard University Press, Cambridge, MA. Reprinted in 1948.

CONI E, BOCCA, B and CAROLI S (1999) Minor and trace element content of two typical Italian sheep dairy products, *Journal of Dairy Research* 66, 589–598.

COWAN RT, ROBINSON JJ, MCHATTIE I and PENNIE K (1981) Effects of protein concentration in the diet on milk yield, change in body composition and the efficiency of utilization of body tissue for milk production in ewes, *Animal Production* 33, 111–120.

CUCCURU C, ZUCCONI A, CARIA A, RUFFO G and CONTINI A (1994) Analysis of risk for somatic cell count in ewes: environmental, management and microbiological factors, *Proceedings of the International Symposium on Somatic Cells and Milk of Small Ruminants*, Bella, Italy, 25–27.

CUCCURU C, PRETI C, MELONI MG and MORONI P (2002) Evaluation of a strategy to reduce milk somatic cell counts in ewes, *Proceeding of the 10th Congress of Mediterranean Federation for Health and Production of Ruminants*, Tunis.

DAVOLI R, DALL'OLIO S and RUSSO V (1985) Polimorfismo delle proteine del latte nella razza ovina delle Langhe [Genetic polymorphism in the proteins of Langhe sheep milk], *Proceedings of the 6th National ASPA (Associazione Scientifica Produzione*

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

*Animale) Congress*, 349–353.

DE BELLIS A (1982) *Il Cacio Pecorino tra Storia e Tradizione* [Pecorino Cheese between History and Tradition], Editori Del Grifo, Montepulciano, Italy.

DE LA FUENTE LF, FERNANDEZ G and SAN PRIMITIVO F (1996) A linear evaluation system for udder traits of dairy ewes, *Livestock Production Science* 45, 171–178.

DE MARIA-GHIONNA C, DELL'AQUILA S and CARINI S (1982) Influenza di una soppressione di una mungitura giornaliera sulla produzione, su talune caratteristiche qualitative e sull'attitudine alla coagulazione del latte in pecore di razza Comisana [Effect of suppressing one milking per day on quality, yield and clotting ability of milk in the Comisana breed of sheep], *Zootecnia e Nutrizione Animale* 8, 407–418.

DE VRIES MJ and VEERKAMP RF (2000) Energy balance of dairy cattle in relation to milk production variables and fertility, *Journal of Dairy Science* 83, 62–69.

DELACROIX-BUCHET A, BARILLET F and LAGRIFFOUL G (1994) Caractérisation de l'aptitude fromagère des laits de brebis Lacaune à l'aide d'un Formagraph [Characterisation of the cheese making aptitude of milk from Lacaune sheep with a Formagraph], *Lait* 74, 173–186.

DENAMUR R and MARTINET J (1961) Action de l'ocytocine sur la sécrétion du lait de brebis [Action of oxytocin on milk secretion in sheep], *Annales d'Endocrinologie* 22, 777–781.

DIEZ-TASCON C, BAYON I, ARRANZ JJ, DE LA FUENTE LF and SAN PRIMITIVO F (2001) Mapping quantitative trait loci for milk production traits on ovine chromosome 6, *Journal of Dairy Research* 68, 389–397.

DIMITROV-IVANOV I and DJORBINEVA M (2003) Assessment of welfare, functional parameters of the udder, milk productive and reproductive traits in dairy ewes of different temperament, *Bulgarian Journal of Agricultural Science* 9, 707–711.

DUDD SN and EVERSHED RP (1998) Direct demonstration of milk as an element of archaeological economies, *Science* 282, 1478–1481.

EMERY RS (1988) Milk fat depression and the influence of diet on milk composition, *Veterinary Clinics of North America: Food Animal Practice* 4, 289–305.

ENNE G (1976) *La Mungitura Meccanica dei Piccoli Ruminanti* [Mechanical Milking of Small Ruminants], CLUED, Milano, Italy.

EPSTEIN H (1985) *The Awassi Sheep with Special Reference to the Improved Dairy Type*, FAO Animal Production and Health Paper 57, FAO, Rome.

EUROPEAN COMMISSION (2003) Commission Directive of 31 October 2003 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed (text with EEA relevance), 2003/100/EC, *Official Journal* L285, 33–37.

EUROPEAN COMMISSION (2006) Commission Regulation of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs (text with EEA relevance), 2006/1881/EC, *Official Journal* L364, 5–24.

EYAL E, LAWI A, FOLMAN Y and MORAG M (1978) Lamb and milk production of a flock of dairy ewes under an accelerated breeding regime, *Journal of Agricultural Science* (Cambridge) 91, 69–79.

FATICHENTI F and FARRIS GA (1973) I lieviti del latte di pecora in Sardegna [Yeasts in sheep milk in Sardinia], *Scienza e Tecnica Lattiero Casearia* 24, 386–390.

FEGEROS K, ZERVAS G, STAMOULI S and APOSTOLAKI E (1995) Nutritive value of dried citrus pulp and its effect on milk yield and milk composition of lactating ewes, *Journal of Dairy Science* 78, 1116–1121.

FERNANDEZ N, DIAZ JR, PERIS C, RODRIGUEZ M, MOLINA MP and TORRES A (1999) Machine

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

milking parameters for the Manchega sheep breed, *Proceedings of the 6th International Symposium on Machine Milking of Small Ruminants*, Athens, 95, 233–238.

FLAM F, CUMLIVSKI B and LAUTNER V (1970) Manufacture of Roquefort-type cheese from ewes' milk. Preliminary communication, *Food Science and Technology Abstracts* 2, 8P1088.

FLAMANT JC and BARILLET F (1982) Adaptation of the principles of selection for milk production to milking ewes: a review, *Livestock Production Science* 9, 549–559.

FLAMANT JC and MORAND-FEHR PC (1982) Milk production in sheep and goats, in IE Coop, *Sheep and Goat Production*, World Animal Science, C.1, Elsevier, Amsterdam, 15, 275–295.

FLEET IR, FULLERTON FM and MEPHAM TB (1986) Effects of exogenous growth hormone on mammary function in lactating Friesland ewes, *Journal of Physiology* 371, 211.

FLEET IR, FULLERTON FM, HEAP RB, MEPHAM TB, GLUCKMAN PD and HART IC (1988) Cardiovascular and metabolic responses during growth hormone treatment of lactating sheep, *Journal of Dairy Research* 55, 479–485.

FOLMAN Y, VOLCANI R and EYAL E (1966) Mother–offspring relationship in the Awassi sheep. I. The effect of different suckling regimes and time of weaning on the lactation curve and milk yield in dairy flocks, *Journal of Agricultural Science* (Cambridge) 67, 359–368.

FOX PF (1993) Cheese: an overview, in PF Fox, *Cheese: Chemistry, Physics and Microbiology*, Chapman & Hall, New York, vol. 1, 1–36.

FREY A, THOMAS VM, ANSOTEGUI R, BURFENING PJ and KOTT RW (1991) Influence of escape protein supplementation to grazing ewes suckling twins on milk production and lamb performance, *Small Ruminant Research* 4, 1–10.

FUERTES JA, GONZALO C, CARRIEDO JA and SAN PRIMITIVO F (1998) Parameters of test day milk yield and milk components for dairy ewes, *Journal of Dairy Science* 81, 1300–1307.

GARDNER RW and HOGUE DE (1964) Effects of energy intake and number of lambs suckled on milk yield, milk composition and energetic efficiency of lactating ewes, *Journal of Animal Science* 23, 935–942.

GARDNER RW and HOGUE DE (1966) Milk production, milk composition and energetic efficiency of Hampshire and Corriedale ewes fed to maintain body weight, *Journal of Animal Science* 25, 789–795.

GARGOURI A, CAJA G, SUCH X, FERRET A, CASALS R and PERIS S (1993) Evaluation of a mixed system of milking and suckling in Manchega dairy ewes *Proceedings of the 5th International Symposium on Machine Milking of Small Ruminants*, *Hungarian Journal of Animal Production* Suppl. 1, 484–499.

GAYA P, MEDINA M and NUÑEZ M (1987) Enterobacteriaceae, coliforms, faecal coliforms and salmonellas in raw ewes' milk, *Journal of Applied Bacteriology* 62, 321–326.

GEENTY KG and DAVISON PG (1982) Influence of weaning age, milking frequency and udder stimulation on dairy milk production and post-partum oestrus interval on Dorset ewes, *New Zealand Journal of Experimental Agriculture* 10, 1–5.

GHANEM I and ORFI M (2009) Aflatoxin M1 in raw, pasteurized and powdered milk available in the Syrian market, *Food Control* 20, 603–605.

GÓMEZ-CORTÉS P, FRUTOS P, MANTECON AR, JUÁREZ M, DE LA FUENTE MA and HERVÁS G (2008) Addition of olive oil to dairy ewe diets: Effect on milk fatty acid profile and animal performance, *Journal of Diary Science* 91, 3119–3127.

GONZALEZ JS, ROBINSON JJ, MCHATTIE I and FRASER C (1982) The effect of source and level

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

of dietary protein on milk yield, and the relationship between the intestinal supply of non-ammonia nitrogen and the production of milk protein, *Animal Production* 34, 31–40.

GONZALO C, ARIZNABARRETA A, CARRIEDO JA and SAN PRIMITIVO F (2002) Mammary pathogens and their relationship with somatic cell count and milk yield losses in dairy ewes, *Journal of Dairy Science* 85, 1460–1467.

GONZALO C, CARRIEDO JA, BLANCO MA, BENEITEZ E, JUÁREZ MT, DE LA FUENTE LF and SAN PRIMITIVO F (2005) Factors of variation influencing bulk tank milk somatic cell count in dairy sheep, *Journal of Dairy Science* 88, 969–974.

GOULAS C, ZERVAS G and PAPADOPOULOS G (2003) Effect of dietary animal fat and methionine on dairy ewes milk yield and milk composition, *Animal Feed Science and Technology* 105, 43–54.

GRIGOROV H and SHALICHEV Y (1962) The effect of number of milkings and of the different intervals between them on the butterfat and protein content of sheep milk obtained. *Proceedings of the XVI International Dairy Congress*, Copenhagen, A 258–264.

HADJIPANAYIOTOU M (1999) Feeding ensiled crude olive cake to lactating Chios ewes, Damascus goats and Friesian cows, *Livestock Production Science* 59, 61–66.

HATFIELD PG, SNOWDER GD, HEAD WA JR, GLIMP HA, STOBART RH and BESSER T (1995) Production by ewes rearing single or twin lambs: effects of dietary crude protein percentage and supplemental zinc methionine, *Journal of Dairy Science* 73, 1227–1238.

HELMER D and VIGNE JD (2007) Was milk a 'secondary product' in the Old World Neolithisation process? Its role in the domestication of cattle, sheep and goats, *Anthropozoologica* 42, 9–40.

HOLCOMBE W, HALLEFORD M and HOEFLER WC (1988) Effects of exogenous ovine growth hormone on reproduction, serum hormone profiles and milk characteristics in early-post-partum fine-wool ewes nursing single or twin offspring, *Theriogenology* 31, 843–854.

HOMER (*circa* 750 BC) *The Odyssey*. Translated by S.O. Andrew, Everyman's Library Dent, London, Dutton, New York, Book 9, 105–119.

HORTON GMJ, WHOLT JE, PALATINI DD and BALDWIN JA (1992) Rumen protected lipid for lactating ewes and their nursing lambs, *Small Ruminant Research* 9, 27–36.

HOUPERT P, FEDERSPIEL B and MILHAUD G (1997) Toxicokinetics of cadmium in lactating and non lactating ewes after oral and intravenous administration, *Environmental Research* 72, 140–150.

HUIDOBRO F (1989) Effects of omitting the Sunday afternoon milking on milk yield of Manchega ewes, *Animal Breeding Abstracts* 57(7), 4929.

HULT K, TEILING A and GATENBACK S (1976) Degradation of ochratoxin A by the ruminant, *Applied and Environmental Microbiology* 32, 443–444.

IANNUZZI L, PERUCATTI A, DI MEO GP, POLIMENO F, CIOTOLA F, INCARNATO D, PERETTI V, CAPUTI-JAMBRENGHI A, PECORARO A, MANNITI F, D'ALESSANDRO A and VONGHIA G (2004) Chromosome fragility in two sheep flocks exposed to dioxins during pasturage, *Mutagenesis* 19, 355–359.

IARC, INTERNATION AGENCY FOR RESEARCH ON CANCER (2002) Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. Summary of data reported and evaluation. *IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans*, 82. International Agency for Research on Cancer, Lyon, France.

JAEGGI JJ, GOVINDASAMY-LUCEY S, BERGER YM, JOHNSON ME, MCKUSICK BC, THOMAS DL and

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

WENDORFF WL (2003) Hard ewe's milk cheese manufactured from milk of three different groups of somatic cell counts, *Journal of Dairy Science* 86, 3082–3089.

JAEGGI JJ, WENDORFF WL, ROMERO J, BERGER YM and JOHNSON ME (2005) Impact of seasonal changes in ovine milk on composition and yield of a hard-pressed cheese, *Journal of Dairy Science* 88, 1358–1363.

JARAMILLO DP, GARCÍA T, BUFFA M, RODRÍGUEZ M, GUAMIS B and TRUJILLO A (2009) Effect of the inclusion of whole citrus in the ration of lactating ewes on the properties of milk and cheese characteristics during ripening, *Journal of Dairy Science* 92, 469–476.

JONES P, SHEARS P, HILLIER D, COMFORT D and LOWELL J (2003) Return to traditional values? A case study of Slow Food, *British Food Journal* 105, 297–321.

KALANTZOPOULOS G (1993) Cheeses from ewes' and goats' milk, in PF Fox, *Cheese: Chemistry, Physics and Microbiology*, Chapman & Hall, New York, vol. 2, 507–553.

KALANTZOPOULOS G (1994) Influence de la présence de cellules somatiques dans le lait sur la qualité des produits laitières, *Proceedings of the International Symposium on Somatic Cells and Milk of Small Ruminants*, Bella, Italy, 25–27 September.

KAN CA and MEIJER GAL (2007) The risk of contamination of food with toxic substances present in animal feed, *Animal Feed Science and Technology* 133, 84–108.

KANIOU-GRIGORIADOU I, ELEFTHERIADOU A, MOURATIDOU T and KATIKOU P (2005) Determination of aflatoxin M1 in ewe's milk samples and the produced curd and Feta cheese, *Food Control* 16, 257–261.

KARAM HA, JUMA KH, AL-SHABIBI M, ELIYA J and ABU-ALMAALI HN (1971) Milk production in Awassi and Hungarian Merino sheep in Iraq, *Journal of Agricultural Science* (Cambridge) 76, 507–511.

KATSIARI MC, VOUTINAS LP and KONDYLI E (2002) Manufacture of yoghurt from stored frozen sheep's milk, *Food Chemistry* 77, 413–420.

KERVINA F, SAGI R, HERMELIN R, GALOVIC B, MÅNSSON S, ROGELJ I, SOBAR B, FRANKEN M and ÖDMAN M (1981) *System solutions for dairy sheep*, Alfa Laval AB, Tumba, Sweden.

KIRICHENKO NS and POPOV GI (1974) Ewe's milk for cheese making, *Moloch Promyshl* 9, 46. Cited by Anifantakis (1986).

KIRK JA, COOPER RA and CHAPMAN A (1984) Effect of shearing housed pregnant ewes on their plasma glucose levels, lamb birth weight and lamb growth rate to 56 days, *Animal Production* 38, 254.

KITESSA SM, PEAKE D, BENCINI R and WILLIAMS AJ (2003) Fish oil metabolism in ruminants. III. Transfer of *n*-3 polyunsaturated fatty acids (PUFA) from tuna oil into sheep's milk, *Animal Feed Science and Technology* 108, 1–14.

KNIGHT TW, BENCINI R, HAACK NA and DEATH AF (1993) Effects of shearing on milk yields and milk composition in machine milked Dorset ewes, *New Zealand Journal of Agricultural Research* 36, 123–132.

LABUSSIÈRE J (1969) Importance, composition et signification des différentes fractions de lait obtenues successivement au cours de la traite mécanique des brebis [Importance, composition and significance of the different fractions of milk obtained during the mechinical milking of sheep], *Annales de Zootechnie* 18, 185–196.

LABUSSIÈRE J (1985) Composition du lait et techniques de traite chez quelques espèces domestiques [Composition of milk and milking techniques in some domestic species], *Bulletin Technique Centre de Recherches Zootechniques et Veterinaires de Theix I.N.R.A.* 61, 49–58.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

LABUSSIÈRE J (1988) Review of physiological and anatomical factors influencing the milking ability of ewes and the organization of milking, *Livestock Production Science* 18, 253–274.

LABUSSIÈRE J, MARTINET J and DENAMUR R (1969) The influence of the milk ejection reflex on the flow rate during the milking of ewes, *Journal of Dairy Science* 36, 191–202.

LANDAU S, KABABYA D, SILANIKOVE N, NITSAN R, LIFSHITZ L, BARAM H, BRUCKENTAL I and MABJEESH SJ (2005) The ratio between dietary rumen degradable organic matter and crude protein may affect milk yield and composition in dairy sheep, *Small Ruminant Research* 58, 115–122.

LINDSAY DR and SKERRITT J (2003) *Improved breeding in dairy goats and milking sheep*. A report for the Rural Industries Research and Development Corporation, RIRDC Publ. No. 02/150.

LOSI G and MARIANI P (1984) Significato tecnologico del polimorfismo delle proteine del latte nella caseificazione a formaggio Grana [Technological significance of milk protein polymorphism in the manufacturing of Grana cheese], *L'Industria del Latte* 20, 23–53.

LUCAS A, ROCK E, CHAMBA JF, VERDIER-METZ I, BRACHET P and COULON JB (2006) Respective effects of milk composition and cheese-making process on the cheese compositional variability in components of nutritional interest, *Lait* 86, 21–41.

LUND T, MIGLIOR F, DEKKERS JCM and BURNSIDE EB (1994) Genetic relationship between clinical mastitis, somatic cell count, and udder conformation in Danish Holsteins, *Livestock Production Science* 39, 243–251.

LYNCH GP, ELSASSER TH, JACKSON C JR, RUMSEY TS and CAMP MJ (1991) Nitrogen metabolism of lactating ewes fed rumen protected methionine and lysine, *Journal of Dairy Science* 74, 2268–2276.

LYSTER RLJ (1972) Review of the progress of dairy science. Section C. Chemistry of milk proteins, *Journal of Dairy Research* 39, 279–318.

MACCIOTTA NPP, MELE M, CAPPIO-BORLINO A and SECCHIARI P (2005) Issues and perspectives in dairy sheep breeding, *Italian Journal of Animal Science* 4, 5–23.

MACLACHLAN DJ and BHULA R (2009) Transfer of lipid-soluble pesticides from contaminated feed to livestock, and residue management, *Animal Feed Science and Technology* 149, 307–321.

MANFREDINI M, CAVANI C, CHIARINI R, SANGUINETTI V and ZARRI MC (1987) Effetti dell'insilato di mais sulle caratteristiche qualitative del latte e del formaggio di pecora [Effects of maize silage on the qualitative characteristics of sheep milk and cheese], *Zootecnia e Nutrizione Animale* 13, 21–28.

MARGETIN M (1994) Present state in sheep breeding in Slovakia with regard to somatic cell counts, *Proceedings of the International Symposium on Somatic Cells and Milk of Small Ruminants*, Bella, Italy, 25–27 September.

MARNET PG and MCKUSICK BC (2001) Regulation of milk ejection and milkability in small ruminants, *Livestock Production Science* 70, 125–133.

MARTIN GB, MILTON JTB, DAVIDSON RH, BANCHERO HUNZICKER GE, LINDSAY DR and BLACHE D (2004) Natural methods for increasing reproductive efficiency in small ruminants, *Animal Reproduction Science* 82–83, 231–245.

MASUCCI F, DI FRANCIA A, ROMANO R, MARESCA DI SERRACAPRIOLA MT, LAMBIASE G, VARRICCHIO ML and PROTO V (2006) Effect of *Lupinus albus* as protein supplement on yield, constituents, clotting properties and fatty acid composition in ewes' milk, *Small Ruminant Research* 65, 251–259.

MAVROGENIS AP and LOUCA A (1980) Effect of different husbandry systems on milk

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

production of purebred and crossbred sheep, *Animal Production* 31, 171–176.

MAYER H (1990) Oxytocin release and milking characteristics of East Friesians and Lacaune dairy sheep, *Sheep Dairy News* (*4th International Symposium on Machine Milking of Small Ruminants*, Tel-Aviv, Israel, 1989) 7, 36–38.

MCKUSICK BC, THOMAS DL and BERGER YM (2001) Effect of weaning system on commercial milk production and lamb growth of East Friesian dairy sheep, *Journal of Dairy Science* 84, 1660–1668.

MCKUSICK BC, THOMAS DL, ROMERO JE and MARNET PG (2002) Effect of weaning system on milk composition and distribution of milk fat within the udder of East Friesian dairy ewes, *Journal of Dairy Science* 85, 2521–2528.

MCKUSICK BC, THOMAS DL and BERGER YM (2003) Effect of omission of machine stripping on milk production and parlor throughput in East Friesian dairy ewes, *Journal of Dairy Science* 86, 680–687.

MCLEAN DM, GRAHAM ERB, PONZONI RW and MCKENZIE HA (1984) Effects of milk protein genetic variants on milk yield and composition, *Journal of Dairy Research* 51, 531–546.

MCLEAN DM, GRAHAM ERB, PONZONI RW and MCKENZIE HA (1987) Effect of milk protein genetic variants and composition on heat stability of milk, *Journal of Dairy Research* 54, 219–235.

MEHENNAOUI S, DELACROIX-BUCHET A, DUCHE A, ENRIQUEZ B, KOLF-CLAUW M and MILHAUD G (1999) Comparative study of cadmium transfer in ewe and cow milks during rennet and lactic curds preparation, *Archives of Environmental Contamination and Toxicology* 37, 389–395.

MELE M, PETACCHI F, BUCCIONI A, SERRA A, FERRUZZI G, ANTONGIOVANNI M and SECCHIARI P (2002) Qualità del latte di pecore Sarde in relazione al contenuto di grasso e di foraggio della dieta [Quality of milk from Sarda sheep in relation to the content of fat and forage in the diet], *Proceedings of the 15th SIPAOC Congress*, Chia Laguna-Cagliari, Italy, 239.

MELE M, BUCCIONI A, PETACCHI F, SERRA A, BANNI S, ANTONGIOVANNI M and SECCHIARI P (2006) Effect of forage/concentrate ratio and soybean oil supplementation on milk yield, and composition form Sarda ewes, *Animal Research* 55, 273–285.

MELE M, SERRA A, CONTE G, POLLICARDO A, DEL VIVA M and SECCHIARI P (2007) Whole extruded linseed in the diet of dairy ewes during early lactation: effect on the fatty acid composition of milk and cheese, *Italian Journal of Animal Science* 6, suppl. 1, 560–562.

MELLOR DJ and MURRAY L (1985) Effect of maternal nutrition on udder development during late pregnancy and on colostrum production in Scottish Blackface ewes with twin lambs, *Research in Veterinary Science* 48, 387–412.

MIKUS M (1970) The influence of hand stripping machine milked ewes on milk composition, *Proceedings of the XVIII International Dairy Congress*, Sydney, Australia, 1E, B.8, 651.

MIKUS M and MASAR M (1978) Milk production and labour productivity in sheep milking twice and thrice daily and without stripping, *Symposium on Machine Milking of Small Ruminants*, Alghero, Italy, 263–276.

MILLS O (1989) *Practical Sheep Dairying: the Care and the Milking of the Dairy Ewe*. Thorsons Publishing, Wellingborough, UK, 1–320.

MORAG M (1968) The effect of varying the daily milking frequency on the milk yield of the ewe and evidence on the nature of the inhibition of milk ejection by half udder milking, *Annales de Zootechnie* 17, 351–369.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

MORAG M, RAZ A and EYAL E (1970) Mother–offspring relationships in Awassi sheep. IV. The effect of weaning at birth, or after 15 weeks, on lactational performance in the dairy ewe, *Journal of Agricultural Science* (Cambridge) 75, 183–187.

MORENO CR, GRUNER L, SCALA A, MURA L, SCHIBLER L, AMIGUES Y, SECHI T, JACQUIET P, FRANÇOIS D, SECHI S, ROIG A, CASU S, BARILLET F, BRUNEL JC, BOUIX J, CARTA A and RUPP R (2006) QLT for resistance to internal parasites in two designs based on natural and experimental conditions of infection, *Proceedings of the 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte, Brazil*.

MORGANTE M, RANUCCI S and CASOLI C (1994) Caratteristiche citologiche del secreto mammario di pecore pluripare in lattazione [Cytological characteristics of the mammary secretion of pluriparous ewes during lactation], *Obbiettivi e Documenti Veterinari* 15, 51–55.

MORGANTE M, BEGHELLI D, PAUSELLI M, DALL'ARA P, CAPUCCELLA M and RANUCCI S (1999) Effect of administration of vitamin E and selenium during the dry period on mammary health and milk cell counts in dairy ewes, *Journal of Dairy Science* 82, 623–631.

MORRISSEY AD, CAMERON AWN and TILBROOK AJ (2008) Artificial lighting during winter increases milk yield in dairy ewes, *Journal of Dairy Science* 91, 4238–4243.

MURRAY TL, BLACHE DB and BENCINI R (2006) Calm dairy ewes produce more milk than nervous dairy ewes, *Proceedings Australian Society of Animal Production* 26, 44.

MURRAY TL, BLACHE DB and BENCINI R (2009) The selection of dairy sheep on calm temperament before milking and its effect on management and milk production, *Small Ruminant Research*, 87, 45–49.

MUSCIO A, CENTODUCATI P, MONTEMURRO O and DELL'AQUILA S (1987) Milk production of grazing Comisana breed sheep fed with or without feed supplementation in dry areas of Southern Italy, *Proceedings of the 2nd International Symposium on the Nutrition of Herbivores*, 195–196.

NUDDA A, PULINA G, VALLEBELLA R, BENCINI R and ENNE G (2000) Ultrasound technique for measuring mammary cistern size of dairy ewes, *Journal of Dairy Research* 67, 101–106.

NUDDA A, BENCINI R, MIJATOVIC S and PULINA G (2002) The yield and composition of milk in Sarda, Awassi and Merino sheep milked unilaterally at different frequencies, *Journal of Dairy Science* 85, 2879–2884.

NUDDA A, MELE M, BATTACONE G, USAI MG and MACCIOTTA NPP (2003) Comparison of conjugated linoleic acid (CLA) content in milk of ewes and goats with the same dietary regimen, *Italian Journal of Animal Science* 2, suppl. 1, 515–517.

NUDDA A, FANCELLU S, PORCU F, BOE F and CANNAS A (2004a) Responses of milk fat composition to dietary non-fiber carbohydrates in Sarda dairy sheep, *Journal of Dairy Science* 87, suppl. 1, 310.

NUDDA A, BATTACONE G, BENCINI R and PULINA G (2004b) Nutrition and milk quality, in G Pulina and R Bencini, *Dairy Sheep Nutrition*, CABI Publishing, Wallingford, UK, 129–149.

NUDDA A, MCGUIRE MA, BATTACONE G and PULINA G (2005) Seasonal variation in conjugated linoleic acid and vaccenic acid in milk fat of sheep and its transfer to cheese and ricotta, *Journal of Dairy Science* 88, 1311–1319.

NUDDA A, BATTACONE G, USAI MG, FANCELLU S and PULINA G (2006) Supplementation with extruded linseed cake affects concentrations of conjugated linoleic acid and vaccenic acid in goat milk, *Journal of Dairy Science* 89, 277–282.

NUÑEZ M, MEDINA M and GAYA P (1989) Ewes' milk cheese: technology, microbiology and

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

chemistry, *Journal of Dairy Research* 56, 303–321.

O'BRIEN E and DIETRICH DR (2005) Ochratoxin A: the continuing enigma, *Critical Reviews in Toxicology* 35, 33–60.

OTHMANE MH, CARRIEDO JA, DE LA FUENTE LF and SAN PRIMITIVO F (2002) Factors affecting test day milk composition in dairy ewes and relationship among various milk components, *Journal of Dairy Research* 69, 53–62.

OWEN JB (1957) A study on the lactation and growth of hill sheep in their native environment and under lowland conditions, *Journal of Agricultural Science* (Cambridge) 48, 387–412.

PALMQUIST DL and JENKINS TC (1980) Fat in lactation rations: review, *Journal of Dairy Science* 63, 1–14.

PALMQUIST DL, BEAULIEU AD and BARBANO DM (1993) Feed and animal factors influencing milk fat composition, *Journal of Dairy Science* 76, 1753–1771.

PARK YW, JUÁREZ M, RAMOS M and HAENLEIN GFW (2007) Physico-chemical characteristics of goat and sheep milk, *Small Ruminant Research* 68, 88–113.

PAUSELLI M, MORGANTE M, CASOLI C, RANUCCI S, DURANTI E and MERHABI H (1992) Caratteristiche del latte ovino in relazione a diversi momenti produtivi e dallo stato sanitario della mammilla, *Proceedings of the 27th Simposio Internazionale di Zootecnia*, 141–157.

PAZZONA A (1980) *Mungitura Meccanica degli Ovini* [Mechanical milking of sheep], Universale Edagricole, Bologna, Italy.

PELL JM, WHEATLEY SD, SIMMONDS AD, THOMAS C, JONES AR and ABBEY JR (1989) Thrice daily milking and exogenous growth hormone treatment: their actions and interaction on lactation in sheep, *Journal of Endocrinology* 121, 251.

PENGOV A (2001) The role of coagulase-negative *Staphylococcus* spp. and associated somatic cell counts in the ovine mammary gland, *Journal of Dairy Science* 84, 572–574.

PENNING PD, ORR RJ and TREACHER TT (1988) Responses of lactating ewes, offered fresh herbage indoors or when grazing, to supplement containing different protein concentration, *Animal Production* 46, 403–415.

PERIS C, DÍAZ JR, BALASCH S, BELTRÁN MC, MOLINA MP and FERNÁNDEZ N (2003) Influence of vacuum level and overmilking on udder health and teat thickness changes in dairy ewes, *Journal of Dairy Science* 86, 3891–3898.

PERUCATTI A, DI MEO GP, ALBARELLA S, CIOTOLA F, INCARNATO D, CAPUTI JAMBRENGHI A, PERETTI V, VONGHIA G and IANNUZZI L (2006) Increased frequencies of both chromosome abnormalities and SCEs in two sheep flocks exposed to high dioxin levels during pasturage, *Mutagenesis* 21, 67–75.

PIOVANO F, SEGHESIO A and GAROFALO G (2005) Igiene della mungitura del latte crudo ovino e caprino [Hygiene of milking raw sheep and goat milk], *Medicina Veterinaria Preventiva* 27, 23–24.

PIRAS M, LIGIOS S, SITZIA M and FOIS N (2007) Out of season sheep milk production in Sardinia, *Proceedings of the 10th National ASPA (Associazione Scientifica Produzione Animale) Congress*, 588–590.

PIREDDA G, PAPOFF CM, SANNAS R and CAMPUS RL (1993) Influenza del genotipo dell'$\alpha_{s1}$-caseina ovina sulle caratteristiche fisico chimiche e lattonimamometriche del latte [Influence of the genotype of sheep $\alpha_{s1}$-casein on the physico-chemical and lacto-dynamometric characteristics of milk], *Scienza e Tecnica Lattiero Casearia* 44, 135–143.

POLLOT GE and GOOTWINE E (2004) Reproductive performance and milk production of

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

Assaf sheep in an intensive management system, *Journal of Dairy Science* 87, 3690–3703.

POLYCHRONIADOU A and VAFOPULOU A (1984) Variations of major mineral constituents of ewe milk during lactation, *Journal of Dairy Science* 68, 147–150.

PORCU F, CANNAS A and RUBATTU R (2003) Urinary and milk excretion of purine derivatives in lactating Sarda ewes, *Proceedings of the 38th International Symposium SIPZOO*, Lodi, Italy, 259–269.

POULTON SG and ASHTON WM (1970) A study of the composition of Clun Forest ewe's milk. IV. The proteins of ewe's milk and their variation with stage of lactation, *Journal of Agricultural Science* (Cambridge) 75, 245–250.

PULINA G (1990) L'influenza dell'alimentazione sulla qualità del latte ovino [The influence of nutrition on the quality of sheep milk], *L'Informatore Agrario* 37, 31–39.

PULINA G and RASSU SPG (1991) Effetto dell'impiego della flavomicina sul contenuto in cellule somatiche nel latte ovino [Effect of flavomycin on the somatic cell count of sheep milk], *Proceedings of the 3rd International Symposium Qualità del Latte Ovino-caprino*, Varese, Italy, I, 193–196.

PULINA G, NUDDA A and CONTU M (1992) Indagine su alcuni fattori di variazione della qualità del latte ovino [Investigation on some factors of variation in the quality of sheep milk], *Proceedings of the SIPAOC Congress on Sheep Milk and Market: Production, Technologies and Marketing*, 29–36.

PULINA G, SERRA A, MACCIOTTA NPP and NUDDA A (1993) La produzione continua di latte nella specie ovina in ambiente mediterraneo [Continuous milk production from sheep in a mediterranean environment], *Proceedings of the 10th National ASPA (Associazione Scientifica Produzione Animale) Congress*, 353–356.

PULINA G, FORBES JM, NUDDA A and BRANDANO P (1994a) Analisi delle correlazioni fra ingestione alimentare, peso corporeo e produzione lattea quanti-qualitativa in pecore di razza Sarda [Analysis of the correlation between food intake, liveweight and yield and quality of milk in Sarda sheep], *Proceedings of the 8th SIPAOC Congress*, 397–400.

PULINA G, NUDDA A, RASSU SPG and BRANDANO P (1994b) Milk secretion during the day and the night in Sarda dairy ewes milked with different intervals, *45th Annual Meeting of the European Association of Animal Production*, Edinburgh, UK, 195.

PULINA G, MACCIOTTA N and NUDDA A (2005) Milk composition and feeding in the Italian dairy sheep, *Italian Journal of Animal Science* 4, 5–14.

PULINA G, NUDDA A, BATTACONE G and CANNAS A (2006a) Effects of the nutrition on the contents of fat, protein, somatic cells, aromatic compounds and undesiderable substances in sheep milk, *Animal Feed Science* 131, 255–291.

PULINA G, MAZZETTE A, BATTACONE G and NUDDA A (2006b) Feed restriction alters milk production traits in Sarda dairy ewes, *Journal of Dairy Science* 89, suppl. 1, 59.

PULINA G, NUDDA A, MACCIOTTA NPP, BATTACONE G, RASSU SPG and CANNAS A (2007) Non-nutritional factors affecting lactation persistency in dairy ewes: a review, *Italian Journal of Animal Science* 6, 115–141.

PURROY A and JAIME C (1995) The response of lactating and dry ewes to energy intake and protein source in the diet, *Small Ruminant Research* 17, 17–24.

PURROY UNANUA A (1986) Machine milking of sheep, *Proceedings of the International Dairy Federation Seminar on Production and Utilization of Ewe's and Goat's Milk*, Athens, Greece. *Bulletin of the International Dairy Federation*, no. 202, 28–41.

RANIERI MS (1993) La variazione dei principali costituenti del latte ovino durante la

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

mungitura [Variation of the principal constituents of sheep milk during milking], *L'Allevatore di Ovini e di Caprini* 10, 14–16.

RASSU SPG, CANNAS EA, NICOLUSSI P, BONELLI P and PULINA G (2006) The impact of machine milking on milk production traits and blood cortisol in primiparous dairy ewes, *Joint Annual Meeting ADSA and ASAS*, Minneapolis, MN, 303.

RASSU SPG, MANCA MG, FRANCESCONI AHD, MEREU A and NUDDA A (2009) Shearing influences the milk fatty acid profile of dairy ewes, *Italian Journal of Animal Science* 8, suppl. 2, 462.

REBAI A, GOFFINET B and MANGIN B (1995) Comparing power of different methods for QTL detection, *Biometrics* 51, 87–99.

REQUENA R, MOLINA P, FERNÁNDEZ N, RODRÍQUEZ M, PERIS C and TORRES A (1999) Changes in milk and cheese composition throughout lactation in Manchega sheep, in F Barillet and NP Zervas, *Milking and Milk Production of Dairy Sheep and Goats*, EAAP Publications no. 95, Wageningen Pers, Wageningen, The Netherlands, 501–506.

REYNOLDS LL and BROWN DL (1991) Assessing dairy potential of Western White-Faced ewes, *Journal of Animal Science* 69, 1354–1362.

RHIND SM, KYLE CE, MACKIE C and TELFER G (2007) Effects of exposure of ewes to sewage sludge-treated pasture on phthalate and alkyl phenol concentrations in their milk, *Science of the Total Environment* 383, 70–80.

RICORDEAU G (1974) Problèmes liés à la finition de la traite à la machine des brebis et des chèvres: importance et intérêt des égouttages machine et manuel et simplification de ces operations [Problems related to the completion of machine milking of sheep and goats: importance and interest of machine and manual stripping and simplification of these operations], *Proceedings of the Symposium sur la Traite Mécanique des Petits Ruminants*, Millau, France. *Annales de Zootechnie*, Numéro Hors Série/1974, 123–131.

ROBINSON JJ, FRASER C, GILL JC and MCHATTIE I (1974) The effect of dietary crude protein concentration and time of weaning on milk production and body weight change in the ewe, *Animal Production* 19, 331–339.

RONCHI B, LACETERA NG, BERNABUCCI U and NARDONE A (1994) Preliminary report on the relationship between selenium status amd milk somatic cell count in selenium deficient Sardinian ewes, *Proceedings of the International Symposium on Somatic Cells and Milk of Small Ruminants*, Bella, Italy, 25–27 September.

ROSSI G and PULINA G (1991) Il ruolo dell'alimentazione nella composizione lipidica del latte ovino [Role of nutrition in the lipidic composition of sheep milk], *L'informatore Agrario* 47, 1–5.

ROSSI G, PULINA G, SERRA A, CANNAS A and BRANDANO P (1991) L'impiego di un alimento unico pellettato nell'alimentazione della pecora da latte: 1 Produzione quanti-qualitativa di latte, livello di ingestione e variazione del peso vivo [Use of a pelleted feed for dairy sheep: 1 Quantity and quality of milk produced, voluntary food intake and live weight variation], *Zootecnia e Nutrizione Animale* 17, 23–34.

ROUSSI V, GOVARIS A, VARAGOULI A and BOTSOGLOU NA (2002) Occurrence of aflatoxin M1 in raw and market milk commercialized in Greece, *Food Additives and Contaminants* 19, 863–868.

ROY NC, FRASER K, LANE GA, SINCLAIR BR and MCNABB WC (2004a) Polyethylene glycol increases intestinal absorption and hepatic uptake of indole and skatole in sheep fed sulla, *Animal Feed Science* 13, 339–342.

ROY NC, FRASER K, LANE GA, REYNOLDS GW, DEIGHTON MH, PETERS JS, SINCLAIR BR, DEATH

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

AF and MCNABB WC (2004b) The effects of condensed-tannins on the net flux of skatole and indole across the mammary gland and their secretion in milk of lactating ewe fed fresh sulla (*Hedysarum coronarium*), *Animal Production* 24, 189–192.

RUIU A and PULINA G (1992) Mastiti ovine, qualità del latte e prospettive di profilassi sanitaria [Ovine mastitis, milk quality and prospects of prevention], *L'Informatore Agrario* 48, 37–40.

RUPP R, SCHIBLER L, CRIBIU E, AMIGUES Y, BOSCHER MY, MURA L, SECHI T, FRAGHI A, CASU S, BARILLET F and CARTA A (2003) Evidence of chromosomal regions controlling somatic cell counts in dairy sheep from two QTL detection projects, *Proceedings of the International Workshop on Major Genes and QTLs in Sheep and Goats*, Toulouse, France, Communication no. 01/45.

RUSSO V and FONTANESI L (2001) Il miglioramento genetico animale: potenzialità dei metodi tradizionali e prospettive della genetica molecolare [Genetic improvement in animals: potential of traditional methods and prospects of molecular genetics], *Zootecnia e Nutrizione Animale* 27, 253–284.

RUSSO V, DAVOLI R and MIGLIORI L (1981) Polimorfismo genetico delle proteine del latte nelle pecore di razza Sarda e Massese [Genetic polymorphism in the proteins of Sarda and Massese sheep milk], *Zootecnia e Nutrizione Animale* 7, 421–428.

SAKUL H and BOYLAND WJ (1992) Evaluation of US sheep breeds for milk production and milk composition, *Small Ruminant Research* 7, 195–201.

SANDLES LD, SUN YX, D'CRUZ AGC, MCDOWELL GH and GOODEN JM (1988) Responses of lactating ewes to exogenous growth hormone: short- and long-term effects on productivity and tissue utilization of key metabolites, *Australian Journal of Biological Sciences* 41, 357–370.

SART S, BENCINI R, BLACHE DB and MARTIN GB (2004) Calm ewes produce milk with more protein than nervous ewes, *Proceedings of the Australian Society of Animal Production* 28, 307.

SCARAMUZZI RJ and MARTIN GB (1984) Pharmacological agents for manipulating oestrus and ovulation in the ewe, in DR Lindsay and DT Pearce, *Reproduction in sheep: Australian Wool Corporation Technical Publication*, Cambridge University Press, Cambridge, 316–325.

SCHECTER A, PAPKE O, TUNG KC, STASKAL D and BIRNBAUM L (2004) Polybrominated diphenyl ethers contamination of United States, *Food Environmental Science and Technology* 38, 5306–5311.

SCHMID P, GUJER E, ZENNEGG M and STUDER C (2003) Temporal and local trends of PCDD/F levels in cow's milk in Switzerland, *Chemosphere* 53, 129–136.

SCHULZ AJ, WIESMULLER T, APPUHN H, STEHR D, SEVERIN K, LANDMANN D and KAMPHUES J (2005) Dioxin concentration in milk and tissues of cows and sheep related to feed and soil contamination, *Animal Physiology and Animal Nutrition* 89, 72–78.

SERRA A, MACCIOTTA NPP, NUDDA A and PULINA G (1993) L'influenza della tecnica di alimentazione per gruppi sulla qualità del latte negli ovini [The influence of the technique of nutrition by groups on the quality of milk in sheep], *Proceedings of the SISVET (Italian Society of Veterinary Science)* 47, 2007–2010.

SERRA FA (1998) La valutazione degli alimenti ed il razionamento negli ovini da latte [Feed evaluation in the formulation of ratios for dairy sheep], PhD thesis, Perugia, Italy.

SEVI A, ANNICCHIARICO G, ALBENZIO M, TAIBI L, MUSCIO A and DELL'AQUILA S (2001a) Effects of solar radiation and feeding time on behavior, immune response and

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

production of lactating ewes under high ambient temperature, *Journal of Dairy Science* 84, 629–640.

SEVI A, TAIBI L, MUSCIO A, ALBENZIO M, DANTONE D and DELL'AQUILA S (2001b) Quality of ewe milk as affected by stocking density and litter treatment with bentonite, *Journal of Food Science* 13, 77–86.

SEVI AM, ALBENZIO R, MARINO A, SANTILLO A and MUSCIO A (2004) Effects of lambing season and stage of lactation on ewe milk quality, *Small Ruminant Research* 51, 251–259.

SHALICHEV Y and TANEV G (1967) Comparative study of the milk yield and composition of Zlatusha sheep, East Friesian sheep and their crosses, *Animal Science* 4, 81–90.

SIGNORET JP (1990) The influence of the ram effect on the breeding activity of ewes and its underlying physiology, in CM Oldham, GB Martin and IW Purvis, *Reproductive Physiology of Merino Sheep. Concepts and Consequences*, School of Agriculture (Animal Science), The University of Western Australia, 59–70.

SIMOS EN, NIKOLAOU EM and ZOIOPOULOS PE (1996) Yield, composition and certain physicochemical characteristics of milk of the Epirus mountain sheep breed, *Small Ruminant Research* 20, 67–74.

STELWAGEN K, GRIEVE DG, WALTON JS, BALL JL and MCBRIDE BW (1993) Effect of prepartum bovine somatotropin in primigravid ewes on mammogenesis, milk production and hormone concentrations, *Journal of Dairy Science* 76, 992–1001.

STUBBS A, ABUD G and BENCINI R (2009) *Dairy Sheep Manual*. Farm Management Guidelines, Rural Industries Research and Development Corporation Publ. No. 08/205.

SUSIN I, LOERCH SC and MCCLURE KE (1995) Effects of feeding a high-grain diet at a restricted intake on lactation performance and rebreeding of ewes, *Journal of Animal Science* 73, 3199–3205.

THOMAS AS, DAWE ST and WALKER RA (1989) Milk protein polymorphism in Hyfer and Border Leicester × Merino sheep, *Milchwissenschaft* 44, 686–688.

THOMAS DL (2004) Overview of the dairy sheep sector in Canada and the United States, *Proceedings of the 10th Great Lakes Dairy Sheep Symposium*, University of Wisconsin–Madison, Department of Animal Science, 166–180.

THOMAS DL, BERGER YM and MCKUSICK BC (1998) Milk and lamb production of East Friesian-cross ewes in northwestern Wisconsin, *Proceedings of the 4th Great Lakes Dairy Sheep Symposium*, University of Wisconsin–Madison, Department of Animal Science, 16–22.

TODARO M, CACCIATORE T, FERRANTELLI M, IRACI C, LETO L, MODICA R, MORTELLARO A, RAGGIO V and SARDINA MT (2004) Influenza del pastazzo di limone sulle caratteristiche qualitative e quantitative del latte di pecora [Influence of citrus pulp on the qualitative and quantitative characteristics of sheep milk], *Proceedings of the 16th SIPAOC Congress*, Siena, Italy, 331.

THOMPSON GE, HARTMANN PE, GOODE JA and LINDSAY KS (1982) Some effects of acute fasting and climatic stresses upon milk secretion in Friesland sheep, *Comparative Biochemistry and Physiology* 70A, 13–16.

TURNER DJ and HUYNH HT (1991) Role of tissue remodelling in mammary epithelial cell proliferation and morphogenesis, *Journal of Dairy Science* 74, 2801–2807.

UBERTALLE A (1989) Il latte di pecora: qualità e fattori di variazione [Sheep milk: quality and factors of variation], *Il Mondo del Latte* 7, 278–288.

UBERTALLE A (1990) Il latte di pecora [Sheep milk: quality and factors of variation], *Atti Accademia Agraria dei Georgofili*, 36, 279–295.

UBERTALLE A and ERRANTE J (1991) Fattori di allevamento influenti sulla qualità del latte

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

ovino [On-farm actors affecting the quality of sheep milk], *Proceedings of the 3rd International Symposium, La Qualità del Latte Ovino-caprino*, 135–158.

UBERTALLE A, ERRANTE J, FORTINA R and AMBROSOLI R (1991) Comportamento reologico e variazioni di alcuni parametri fisico-chimici e biologici del latte ovino [Rheological behaviour and variations of some physico-chemical and biological parameters of sheep milk], *Proceedings of the 9th National ASPA (Associazione Scientifica Produzione Animale) Congress*, 999–1008.

UCEDA R, GUILLEN AM, GAYA P, MEDINA M and NUÑEZ M (1994a) The effect of ewe milk lactoperoxidase system on *Pseudomonas fluorescens* growth, casein breakdown, peptide formation and milk coagulation characteristics, *Milchwissenschaft* 49, 139–143.

UCEDA R, PICON A, GUILLEN AM, GAYA P, MEDINA M and NUÑEZ M (1994b) Characteristics of Manchego cheese manufactured from ewe raw milk preserved by addition of carbon dioxide or by activation of the lactoperoxidase system, *Milchwissenschaft* 49, 678–683.

UPADHAYA SD, SUNG HG, LEE CH, LEE SY, KIM SW, CHO KJ and HA JK (2009) Comparative study on the aflatoxin B1 degradation ability of rumen fluid from Holstein steers and Korean native goats, *Journal of Veterinary Science* 10, 29–34.

VARRO MT (35 BC) Of milk and wool, in MT Varro, *De Re Rustica [On Agriculture]*. Book II, X.II–XI.3.

VASTA V, NUDDA A, CANNAS A, LANZA M and PRIOLO A (2008) Alternative feed resources and their effects on the quality of meat and milk from small ruminants – a review, *Animal Feed Science and Technology* 147, 223–246.

VIALLON C, MARTIN B, VERDIER-METZ I, PRADEL P, GAREL JP, COULON JB and BEDAGU JL (2000) Transfer of monoterpenes and sesquiterpenes from forages into milk fat, *Lait* 80, 635–641.

VIPOND JE, KING ME and INGLIS DM (1987) The effect of winter shearing of housed pregnant ewes on food intake and animal performance, *Animal Production* 45, 211–221.

VOLANIS M, ZOIOPOULOS P and TZERAKIS K (2004) Effects of feeding ensiled sliced oranges to lactating dairy sheep, *Small Ruminant Research* 53, 15–21.

VOUTSINAS LP, DELEGIAMIS C, KATSIARI MC and PAPPAS C (1988) Chemical composition of Boutsiko ewe milk during lactation, *Milchwissenschaft* 43, 766–771.

VRECL M, URSIC M, POGACNIK A, ZUPANCIC-KRALJ L and JAN J (2005) Excretion pattern of co-planar and non-planar tetra- and hexa-chlorobiphenyls in ovine milk and faeces, *Toxicology and Applied Pharmacology* 204, 170–174.

WARD RJ, TRAVERS MT, VERNON RG, SALTER AM, BUTTERY PJ and BARBER MC (1997) The ovine stearoyl-CoA desaturase gene: cloning and determinationof gene number within the ovine genome, *Biochemical Society Transactions* 25, S673.

WHEELER JL, REARDON TF and LAMBOURNE LJ (1962) The effect of pasture availability and shearing stress on herbage intake of grazing sheep, *Australian Journal of Agricultural Research* 14, 364–372.

WILDE CJ and PEAKER M (1990) Autocrine control of milk secretion, *Journal of Agricultural Science* (Cambridge) 114, 235–238.

WILDE CJ, KNIGHT CH and PEAKER M (1996) Autocrine regulation of milk secretion, in CJC Phillips, *Progress in Dairy Science*, CAB International, London, 311–332.

WILLETT LB, O'DONNELL AF, DURST HI and KURZ MM (1993) Mechanisms of movement of organochlorine pesticides from soils to cows via forages, *Journal of Dairy Science* 76, 1635–1644.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

WILSON R, ZIPRIN R, RAGSDALE S and BUSBEE D (1985) Uptake and vascular transport of ingested aflatoxin, *Toxicology Letters* 29, 169–176.

WODZICKA-TOMASZEWSKA M (1964) The effect of shearing on the appetite of two-tooth ewes, *New Zealand Journal of Agricultural Research* 7, 654–662.

XIAO H, MARQUARDT RR, FROHLICH AA, PHILLIPS GD and VITTI TG (1991) Effect of a hay and a grain diet on the bioavailability of ochratoxin A in the rumen of sheep, *Journal of Dairy Science* 69, 3715–3723.

YIANNIKOURIS A and JOUANY JP (2002) Mycotoxins in feeds and their fate in animals: a review, *Animal Research* 51, 81–99.

ZENOU A and MIRON J (2005) Milking performance of dairy ewes fed pellets containing soy hulls as starchy grain substitute, *Small Ruminant Research* 57, 187–192.

ZERVAS G, FEGEROS K, KOYTSOTOLIS K, GOULAS C and MANTZIOS A (1998) Soy hulls as a replacement for maize in lactating dairy ewe diets with or without dietary fat supplements, *Animal Feed Science and Technology* 76, 65–75.

ZHANG RH, MUSTAFA AF and ZHAO X (2006) Effects of feeding oilseeds rich in linoleic and linolenic fatty acids to lactating ewes on cheese yield and on fatty acid composition of milk and cheese, *Animal Feed Science and Technology* 127, 220–233.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

# 14

# Improving buffalo milk

**M. Guo, University of Vermont, USA and G. Hendricks, University of Massachusetts, USA**

IP Address: 129.132.208.100

**Abstract:** Buffalo are the second largest source of milk supply in the world; world production of buffalo milk exceeds 75 million metric tons per year and is increasing steadily at about 3% per year. Dairy cattle produce 84% of the total milk in the world with an average fat and protein content of 4% and 3.5%, respectively, while the average fat content of buffalo milk is about 8% and the protein content in buffalo milk ranges from 4% to almost 4.5%. Thus, in terms of energy-corrected milk, buffalo milk accounts for about twice the food contribution implied by the volume of buffalo milk produced yearly.

Physiologically, buffalo are capable of breeding throughout the year and having a calf every year. However, oestrus detection in buffalo is a problem; while irregular oestrus, and anoestrus lasting three or four months, are rarely seen in modern Western dairy herds, it is a common problem in small, rural buffalo herds, which makes up the majority of the buffalo production in most of the world.

There are several important herd management factors that should be considered to improve milk production in buffalo. These include managing the nutritional status of the dam around calving, pre- and post-partum hygiene, good milking management, balanced feeding throughout the year, oestrus detection and artificial insemination, and finally managing thermal stress and improving housing.

Buffalo milk can be utilized for manufacture of a wide variety of dairy products. Because of the differences in compositional and physicochemical properties between buffalo and bovine milk, processing technology and equipment designed for cow's milk may not be suitable for buffalo milk processing. Modifications must be considered for making certain products.

**Key words:** buffalo, management, milk, composition, products.

© Woodhead Publishing Limited, 2010

## 14.1 Introduction

World milk production has doubled in the last few decades and it is noteworthy that currently buffalo supply about 12% of the total world milk production. Table 14.1 shows buffalo distribution and milk production among the major buffalo farming countries. India and Pakistan have produced, respectively, 60% and 30% of the world's buffalo milk. In India, buffalo milk contributes 55%, and in Pakistan 75%, of their total milk production (Högberg, 2004). The Food and Agriculture Organization of the United Nations estimates that the world's milk production from buffalo is about 75 million metric tons yearly, most of which is produced in Asia and the Pacific nations (73 million metric tons) by low-producing animals (FAO, 2007). Probably a large portion goes unreported as buffalo milk is largely handled by small rural family farms for their own use. For comparison, the FAO estimates the world's milk production from cows at 460 million metric tons, about six times that of buffalo production. Buffalo milk is used in much the same way as cow's milk. It is high in fat and total solids, which gives it a rich flavor. Many people prefer it to cow's milk and are willing to pay more for it. The demand for buffalo milk in India (about 60% of the milk consumed and over 80% in some states) is reflected in the prices paid for a liter of milk: about 130 paisa for cow's milk compared with about 200 paisa for buffalo milk. Although the two milks are roughly nutritionally equivalent, customers are willing to pay 54% more for buffalo milk. In the US, buffalo milk and its products are also considered as specialties. In Egypt the severe mortality rate among buffalo calves is due in part to the sale of buffalo milk, which is in high demand, thus depriving calves of proper nourishment. This also occurs throughout India and Pakistan where in the Bombay area alone an estimated 10,000 newborn calves starve to death each year because of lack of milk (Kay, 1974; Jainudeen, 2003).

There are two species of buffalo: the African Buffalo (*Syncerus*), which are wild, and the Asian Buffalo (*Bubalus*), which for the most part are domesticated (*Bubalus bubalis*). Within the Asian Buffalo there are two distinct types: swamp and river buffalo (Toll and Halnan, 1976). Swamp buffaloes tend to be indigenous to those parts of Asia in which there is not a large base of consumers of fresh drinking milk or milk-based products. This covers Indonesia northwards to China. River buffaloes tend to be found in those countries where milk plays a more

IP Address: 129.132.208.100

**Table 14.1** Number of domestic buffalo and milk production among major buffalo farming countries

| Country | Number (× 1000) | Milk (× 1000 tons) |
|---|---|---|
| India | 92,090 | 35,340 |
| Pakistan | 21,213 | 16,456 |
| China | 20,818 | 2,300 |
| Nepal | 3,419 | 729 |

Source: adapted from Jainudeen (2003).

© Woodhead Publishing Limited, 2010

important part in the human diet. They range from India through Pakistan into the Middle East, through the Caucasus and into the Balkans. River buffalo are primarily a triple-purpose animal, producing milk, meat and draft power, while the swamp buffalo are kept mainly for meat and draught. Buffaloes vary greatly in size. The single greatest reason for this variation is environmental. In areas where animal feed is scarce the animals tend to be smaller than in those areas where forage is more plentiful. Typically, adult females can range anywhere between 350 kg high in the Himalayas to 800 kg in Bulgaria and Italy (Cockrill, 1974).

Twelve of the 18 major breeds of water buffalo are kept primarily for milk production (although males may be used for draft and all animals are eventually used for meat). The main milking breeds of India and Pakistan are the Murrah, Nili/Ravi, Surti, Mehsana, Nagpuri, and Jafarabadi. The buffalo of Egypt, Eastern Europe (Bulgaria, Romania, Yugoslavia, and some parts of the former USSR) and Italy are used primarily for milk production and there are also herds used principally for this purpose in Iran, Iraq, and Turkey (Cockrill, 1974). Buffalo milk is commercially more viable than cow milk for the manufacture of both fat-based and fat-free milk products, such as butter, ghee and milk powders, because of its lower water content and higher fat content. By virtue of the greater opacity of casein micelles, coupled with higher levels of colloidal proteins, calcium and phosphorus, buffalo milk is more densely white and has superior whitening properties as compared to cow milk (Cockrill, 1974). Therefore, unlike cows' milk (which is pale and creamy-yellow in color) and cow milk fat (which is golden yellow in color), buffalo milk is distinctively whiter. Processed buffalo milk and cream are intrinsically whiter and more viscous than their cow milk counterparts, because of conversion of greater levels of calcium and phosphorus into the colloidal form. Buffalo milk is, therefore, more aptly suitable for the production of tea and coffee whiteners than cow milk. Higher innate levels of proteins and fat render buffalo milk a more economically attractive alternative to cow milk for the production of casein, caseinates, whey protein concentrates and a wide range of fat-rich dairy products (Cockrill, 1974).

IP Address: 129.132.208.100

## 14.2 Chemical composition

Buffalo milk contains less water, more total solids, more fat, slightly less lactose, and more protein than cow's milk (see Table 14.2). It seems thicker than cow's milk because it generally contains more than 16% total solids compared to 12–14% for cow's milk (Addeo *et al.*, 1977). In addition, its fat content is usually 50–60% (or more) higher than that of cow's milk. Although the butterfat content is usually 6–8%, it can range as high as 12% in the milk of some well-fed dairy buffalo herds in the US, but the butterfat content can run well below 5% in the milk of swamp buffalo of East Asia.

Because of its high butterfat content, buffalo milk has considerably higher energy value than cow's milk. Phospholipids are lower but saturated fatty acids are higher in buffalo milk. Studies have shown that digestibility is not adversely

© Woodhead Publishing Limited, 2010

**Table 14.2** Physicochemical properties of buffalo and bovine milk

| | Buffalo | Bovine |
|---|---|---|
| *Gross composition (%)* | | |
| Fat | 7.3 | 3.7 |
| Lactose | 4.4 | 4.8 |
| Protein | 4.5 | 3.4 |
| Total solids | 17.2 | 12.7 |
| Ash | 0.9 | 0.7 |
| Titratable acidity | 0.16 | 0.15 |
| pH | 6.8 | 6.7 |
| *Protein profile (g/100 ml)* | | |
| $\alpha_{s1}$-casein | 1.6 | 1.3 |
| $\alpha_{s2}$-casein | 0.25 | 0.35 |
| $\beta$-casein | 1.4 | 1.0 |
| $\kappa$-casein | 0.45 | 0.3 |
| $\beta$-lactoglobulin | 0.4 | 0.3 |
| $\alpha$-lactalbumin | 0.14 | 0.13 |
| *Fatty acid profile (%)* | | |
| C4 | 3.9 | 3.3 |
| C6 | 1.6 | 1.0 |
| C8 | 0.8 | 1.3 |
| C10 | 1.6 | 3.0 |
| C12 | 2.0 | 3.1 |
| C16 | 33 | 28 |
| C18:0 | 12 | 15 |
| C18:1 | 25 | 28 |
| C18:2 | 1.5 | 1.9 |
| CLA[a] | 0.6 | 0.5 |
| *Selected minerals (mg/100 ml)* | | |
| Calcium | 180 | 120 |
| Magnesium | 20 | 13 |
| Sodium | 45 | 50 |
| Potassium | 100 | 140 |
| Phosphate | 88 | 75 |
| Chloride | 64 | 120 |
| Zinc | 0.4 | 0.3 |
| Iron | 0.08 | 0.04 |
| Copper | 0.01 | 0.01 |
| *Selected vitamins* | | |
| Vitamin A (IU/100 ml) | 3200 | 1500 |
| Tocopherol (mg/100 ml) | 33 | 31 |
| Thiamine ($\mu$g/100 ml) | 35 | 30 |
| Riboflavin ($\mu$g/100 ml) | 160 | 170 |
| Ascorbic acid (mg/100 ml) | 6.7 | 2.0 |

[a] Conjugated linoleic acid (Guo, 2003).
Source: based on Guo (1993) and Sahai (1996).

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

affected by this. Because of the high fat content, the buffalo's total fat yield per lactation compares favorably with that of improved breeds of dairy cattle; it is much higher than that of most of the indigenous cows of Asia and the Pacific regions.

Normally the protein in buffalo milk contains more casein and slightly more albumin and globulin than cow's milk. Several researchers have claimed that the biological value of buffalo milk protein is higher than that of cow's milk, but this has not been clinically proven (Patel and Mistry, 1997). The proteins of buffalo milk, in particular the whey proteins, are much more heat stable than those of cow's milk. Dried milk products prepared from buffalo milk exhibit higher levels of undenatured proteins when processed under similar conditions (Patel and Mistry, 1997), due probably to the milk's higher total solids. The mineral content of buffalo milk is nearly the same as that of cow's milk except for phosphorus, calcium, and iron, which occur in roughly twice the amounts in buffalo milk. Buffalo milk tends to be lower in salt. There is a significantly lower water content and a higher fat content in buffalo milk than in cow's milk, with almost 45% more milk solids in buffalo milk. Because of this, buffalo milk is better suited for the manufacture of fat-based milk products, such as butter, ghee, and milk powder. And even with almost twice the fat content of cow's milk, buffalo milk has only a fraction of the cholesterol ($0.65\,mg\,g^{-1}$ vs. $3.13\,mg\,g^{-1}$) (see Patel and Mistry, 1997). Buffalo milk contains relatively higher levels of conjugated linoleic acid (CLA) than cow's milk. This may make buffalo's milk a more nutritionally wise and health-conscious choice for dairy product consumers. However, the energy density of buffalo milk is much higher than that of cow's milk.

Buffalo milk also lacks the yellow pigment carotene, precursor for vitamin A, and its whiteness is frequently used to differentiate it from cow's milk in the market. Despite the absence of carotene, the vitamin A content in buffalo milk is almost as high as that of cow's milk. Apparently the buffalo converts the carotene in its diet to vitamin A (Patel and Mistry, 1997). The two milks are similar in B complex vitamins and vitamin C, but buffalo milk tends to be lower in riboflavin.

IP Address: 129.132.208.100

## 14.3 Milk products

Buffalo milk, like cow's milk, can be utilized for the manufacture of a wide variety of dairy products such as cream, butter, butter oil (clarified butter or ghee), UHT cream, ice cream, yogurt and some cheeses without changing the equipment or processing strategies. However, processing technology and equipment designed for cow milk product manufacture are often not adequately suited for buffalo milk processing due to the differences in compositional, physicochemical and functional properties between buffalo and bovine milk. The following considerations need to be taken into account in buffalo milk product processing (Sahai, 1996):

© Woodhead Publishing Limited, 2010

- Buffalo milk variations are greatly affected by age, stage of lactation, season, and breed.
- Buffalo milk has higher total solids, fat content and calcium level. These will affect processing and yield of certain products.
- Casein micelles are larger and there is a higher level of calcium in buffalo milk than in cow milk. Buffalo milk's rennet clotting time is much less and its curd tension is higher than that of cow milk.
- The buffering capacity, pH and viscosity of buffalo milk are higher than those of cow milk.
- The fermentation and/or ripening process of buffalo milk is generally slower.
- Butter made from buffalo milk is harder than that of cow milk due to its higher levels of saturated fatty acids.

Buffalo are the second largest (after cows) source of milk in the world. They are of great economic importance in India in preparing 'toned' milk – a mixture of buffalo milk and milk made by reconstituting dry skim-milk powder. The richness of buffalo milk makes it highly suitable for processing if proper processing technologies are exploited. To produce 1 kg of cheese, a cheese maker requires 8 kg of cow's milk but only 5 kg of buffalo milk. To produce 1 kg of butter requires 14 kg of cow's milk but only 10 kg of buffalo milk. Because of these high yields, processors appreciate the value of buffalo milk.

As a specialty product, buffalo cheese is highly prized for its pure white appearance and smooth texture. In many countries it is among the most desirable cheeses (Mozzarella and Ricotta in Italy, Gemir in Iraq, the salty cheeses of Egypt, and Pecorino in Bulgaria, for example). In Venezuela all the cheese produced from the small La Guanota milking herd in the Apure River basin (about 100 kg a day) is bought by the Hilton Hotel Caracas and sells for twice the price of cheese made from cow's milk (Falvey, 1999).

IP Address: 129.132.208.100

Cheeses, including buffalo milk cheeses, are becoming increasingly popular throughout the world. Demand is rising at a rate that is among the highest for any food product. Cheese offers particular benefit to areas where refrigeration is not widely available, where transporting high-protein foods to remote areas is difficult, and where seasonal fluctuations affect milk supplies. Buffalo milk may make cheesemaking profitable on an even smaller scale than conventional dairying; it is more concentrated than cow's milk and requires relatively less energy to transport and process (an increasingly important factor where fuels are limited) (Falvey, 1999). Although much in demand for making soft cheese, buffalo milk is less desirable for making hard cheeses such as Cheddar or Gouda. During cheesemaking, buffalo milk produces acid more slowly than cow's milk, retains more water in the curd, and loses more fat in the whey. However, some of these problems can be eliminated by reducing the calcium content of the buffalo milk through ionic exchange with sodium chloride (Czulak, 1964; Czulak *et al.*, 1969). Ultrafiltration also appears to help reduce some of the typically observed problems during cheese making with buffalo milk (Patel and Mistry, 1997).

© Woodhead Publishing Limited, 2010

## 14.4 Dairy management and milk production

The characteristics of the dairy buffalo so closely approximate those of the dairy cow that successful methods of breeding, husbandry, and feeding for milk production for the cow can be applied equally to the dairy buffalo (Jainudeen, 2003). Buffalo are generally very docile and easy to handle. They rarely show aggression to people but can be very aggressive to one another. In general the husbandry of buffalo is not dissimilar to that of cattle. However, buffalo have not been systematically bred for uniform udders and teat size; therefore it is difficult to milk buffalo using modern milking machines (Aliev, 1969, 1970; Alim, 1982). Nevertheless, thousands of buffalo are machine milked in Bulgaria and Italy, so machine milking can be adapted to buffalo and vice versa. Buffalo in the research herd at Ain Shams University in Egypt have adapted well to machine milking (Badran, 1992). Machine milking is a standard practice at the university's buffalo farming operation. The calves are separated from their dams immediately after birth and no problems of milk letdown have been observed (Badran, 1992). However, milk letdown is often an issue in small rural milking farms and some buffalo dams have problems with milk letdown without a calf around. Frequently, a calf is kept with the cow and is tied to her foreleg at milking time. In India, Burma, and other Asian countries a dummy calf may be provided at milking (Kay, 1974).

The lactation length is on average 300 days for the Murrah breed, and about 20 days shorter for the river and swamp buffalo (Aliev, 1969). Milk yields typically range from 1500 to 1800 kg for the first lactation with a steady increase to a peak during the fourth. This peak level is usually maintained through the ninth lactation, at about 16 years of age (Kay, 1974; Lall and Narayanan, 1991). Modern dairy cows in the US will continue to be productive members of the herd for 10 or more lactation cycles, with average milk yields of 8800 kg of milk or more (different breeds produce within a range of around 4000 to over 10,000 kg of milk per annum). However, average herd life for the higher production breeds is only four years due to problems associated with infertility, diseases, lameness, or production (NRC, 1988). Some animals, despite their high genetic potential, simply fail to produce economic levels of milk to justify their feed costs. Just as with dairy cows, buffalo herd life is strongly correlated with production levels (Lall and Narayanan, 1991). Lower production cows live longer than high production cows, but are arguably less profitable because of higher feed cost to maintain high production levels. However, the vast majority of buffalo milk is produced by smallholder farms and the buffalo are also draft animals, unlike dairy cattle in the US. Therefore, it is not uncommon for small family farms to keep lower producing buffalo because of the high cost of replacing them (Falvey, 1999).

In countries like India and Egypt, the milk yield of dairy water buffalo is generally higher (680–800 kg) than for local cattle (360–500 kg) (Badran, 1992). However, since selection for exceptional milk production is not conducted systematically, large variations in yield occur between individual animals, and

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

milk production of buffalo falls short of its potential. Nonetheless, some outstanding yields have been recorded. One Indian government farm reported average yields for milking buffalo from 4 to 7 kg per day in lactations averaging 285 days. Daily yields of 12 kg have been reported for some Bulgarian buffalo cows and a daily production of over 20 kg has been reported for some remarkable animals in India. A peak milk yield of 31.5 kg in a day has been recorded from a champion Murrah buffalo in the All India Milk Yield Competition conducted by the Government of India (Lall and Narayanan, 1991). At Caserta, Italy, a herd of 1600 machine-milked, pedigreed dairy buffaloes has produced average yields of 1500 kg during lactations of 270 days (5.5 kg per day per buffalo). In Pakistan and India averages of 6.8 kg and 6.3 kg per day are common for Nili/Ravi buffalo cows and Murrah buffalo cows during lactations averaging 282 days (Williamson and Payne, 1965; Cady *et al.*, 1983).

Swamp buffalo of Southeast Asia are generally considered poor milk producers. They are mainly used as draft animals, but it may be that their milk potential has been underestimated due to the poor quality of feed; most subsist on whatever they can graze on while working and don't receive any supplemental feed. In the Philippines, swamp buffalo cows with nursing calves have produced 300–800 kg of milk during lactation periods of 180–300 days (Falvey, 1999). In Thailand, swamp buffaloes selected and reared for milk production have yielded 3–5 kg per day during 305-day lactations. As with cattle, the percentages of fat, protein, and total solids decrease as the milk yield increases (NRC, 1988).

IP Address: 129.132.208.100

## 14.5 Feeding management

India, Pakistan and most Asian countries have limited feed resources for feeding their buffalo stock (Whyte and Mathur, 1965a, 1965b). The available resources are essentially tropical pasture, straw and crop residue, all generally low in protein (Jainudeen, 2003). As buffalo are capable of surviving on very little, they are often fed very little. The energy and protein requirements for lactating buffalo are on the order of 100 times higher than for maintenance (1.28 g/kg body weight vs. 126.6 to 166.34 g/kg body weight during lactation). There is no physiological need to feed concentrate to maintain butterfat content in buffalo milk, since buffalo release excess fat into the milk and store only a minimum in body tissues (Jainudeen, 2003), although there is ample evidence to suggest that feed supplements do improve milk yield. Like dairy cattle, buffalo fed supplements show better body condition, shorter calving intervals and higher milk yields (Jainudeen, 2003). Even a simple block lick of urea and molasses supplies fermentable energy and macro- and micro-minerals to make rumen microflora and fauna more efficient in fermenting and digesting the low quality roughage available as feedstock. However, there are several physiological factors that contribute to the buffalo's ability to utilize poor quality roughage and crop residue better than cows. Among these are the buffalo's large rumen volume,

© Woodhead Publishing Limited, 2010

very high rate of salivation, slower passage of digestate through the reticulo-rumen, slow rumen motility and high cellular activity. The buffalo's physical and physiological characteristics make it better suited than the native cows as a dairy animal in these regions of the world (Williamson and Payne, 1965).

## 14.6 Factors that influence the yield and composition of buffalo milk

Presently, there are 18 recognized breeds of buffalo. All vary in milk yield and composition. However, just as with cow's milk, buffalo milk composition is affected by many factors including breed, age, stage of lactation and feeding regime. Table 14.3 shows the gross composition of milk from different buffalo breeds. Buffalo allowed a dry period of 50 days or shorter between oestrus and breeding produce less milk in the following lactation (Williamson and Payne, 1965). And just as with cow's milk, gross composition of buffalo milk changes with lactational age, with increasing protein and fat, while lactose decreases with advancing lactation (Lall and Narayanan, 1991). Even the composition of the milk fat and protein changes with advancing stage of lactation (Table 14.4). Large milk fat globules increase in percentage, short chain fatty acids decrease while long chain and unsaturated fatty acids increase, and the concentration of cholesterol increases because of the breakdown of the epithelial cells that line the mammary ducts of the udder (Hofi *et al.*, 1977).

A buffalo is capable of breeding throughout the year and having a calf every year. They carry their calf for 10 months; twin calves and calving difficulties are very rare. Buffalo are very hardy and tolerant of weather conditions (Tailor and Jain, 1993). They are extremely disease resistant. Calves especially rarely suffer from pneumonia or non-nutritional scours. Lameness and clinical mastitis are also rare in adults (Kay, 1974; Saini *et al.*, 1994). Milk yields in buffalo herds are usually recorded over a period of 305 days from calving. Where feeding is adequate and the incidence of disease is controlled, animals will frequently continue to give satisfactory daily yields well beyond this period.

A serious handicap in the development of superior milking characteristics of the buffalo is that artificial insemination (AI) often presents particular difficulties. Oestrus detection in buffalo is a problem; irregular oestrus, and anoestrus lasting three or four months, are rarely seen in modern Western dairy herds.

**Table 14.3** Comparative gross composition (%) of buffalo's and cow's milk

| Animal | Total solids | Protein | Fat | Lactose |
|---|---|---|---|---|
| Murrah buffalo | 17.96 | 4.36 | 7.45 | 4.83 |
| Swamp buffalo | 18.34 | 4.13 | 8.95 | 4.78 |
| Indian native cow | 12.15 | 3.25 | 3.60 | 4.59 |
| Modern US dairy cow | 12.50 | 3.40 | 3.50 | 5.00 |

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

**Table 14.4** Changes in chemical composition (%) of commingled water buffalo milk during a year

| Component | Jan | Feb | Mar | Apr | May | Jun |
|---|---|---|---|---|---|---|
| pH | 6.79 ± 0.01 | 6.76 ± 0.01 | 6.82 ± 0.01 | 6.98 ± 0.01 | 6.88 ± 0.01 | 6.85 ± 0.01 |
| Total solids | 18.45 ± 0.04 | 18.20 ± 0.09 | 18.48 ± 0.03 | 16.39 ± 0.01 | 17.51 ± 0.03 | 16.61 ± 0.02 |
| Fat | 7.63 ± 0.03 | 6.58 ± 0.03 | 7.07 ± 0.06 | 6.68 ± 0.03 | 6.90 ± 0.01 | 6.80 ± 0.01 |
| Crude protein | 5.37 ± 0.11 | 5.23 ± 0.03 | 5.25 ± 0.02 | 4.65 ± 0.06 | 5.12 ± 0.04 | 4.59 ± 0.37 |
| Lactose | 4.50 ± 0.08 | 4.73 ± 0.04 | 4.59 ± 0.10 | 4.49 ± 0.06 | 4.56 ± 0.03 | 4.50 ± 0.07 |
| Ash | 0.92 ± 0.01 | 0.92 ± 0.03 | 0.92 ± 0.01 | 0.91 ± 0.05 | 0.91 ± 0.02 | 0.91 ± 0.03 |
| Component | Jul | Aug | Sept | Oct | Nov | Dec |
| pH | 6.82 ± 0.01 | 6.89 ± 0.01 | 6.91 ± 0.01 | 6.91 ± 0.01 | 6.78 ± 0.03 | 6.83 ± 0.03 |
| Total solids | 17.29 ± 0.08 | 17.78 ± 0.01 | 17.79 ± 0.01 | 17.98 ± 0.02 | 18.47 ± 0.01 | 18.40 ± 0.03 |
| Fat | 6.57 ± 0.06 | 7.02 ± 0.03 | 7.40 ± 0.01 | 7.60 ± 0.01 | 7.97 ± 0.06 | 7.37 ± 0.06 |
| Crude protein | 4.70 ± 0.09 | 5.14 ± 0.10 | 5.11 ± 0.13 | 5.10 ± 0.07 | 4.98 ± 0.26 | 4.94 ± 0.02 |
| Lactose | 4.60 ± 0.08 | 4.49 ± 0.02 | 4.55 ± 0.04 | 4.66 ± 0.06 | 4.69 ± 0.01 | 4.70 ± 0.03 |
| Ash | 0.92 ± 0.01 | 0.92 ± 0.04 | 0.92 ± 0.03 | 0.92 ± 0.01 | 0.92 ± 0.02 | 0.92 ± 0.05 |

Source: Guo (2003).

Large-scale dairy buffalo production is a greater reality in India and Pakistan than anywhere else in the world, even though it represents less than 2% of the buffalo farms in those two countries. Further, because most buffalo milk still comes from small farms, there is a lack of organization around selective breeding programs for the best milking characteristics (Chantalakhana, 1999; Falvey, 1999). AI is still practiced only to a very limited extent for buffalo breeding and herd management: in Italy for 2.5% of the buffalo, in Egypt and Iran for 0.5%, and in Romania for 0.1% of animals. In the large cooperative and state farms in Bulgaria, AI is used on 80% of the buffalo. In most other countries it is not used at all. The low use of AI has slowed down the implementation of selection schemes for genetic improvement of milk and meat productivity. Research trials conducted in Italy have indicated that artificial insemination is feasible and successful in buffalo using techniques to synchronize oestrus and boost fertility (Mason, 1974). Where there has been selection over a number of generations combined with improved nutrition, controlled environmental conditions and protection from disease, high lactation yields from individuals and small herds of animals have been achieved (Lall and Narayanan, 1991). It was estimated as far back as the 1960s that the milk requirements for all of India could be met by about half the present number of cows and buffalo, with further savings of available feedstocks that are presently being wasted on uneconomic animals if India were to adopt a nationwide program using modern animal husbandry practices including AI to produce improved animal stocks (Whyte and Mathur, 1965a, 1965b; Nanda and Nakao, 2003).

IP Address: 129.132.208.100

## 14.7 Factors to consider for improving milk production and reproductive capacity of buffalo

Buffalo account for about 80 million metric tons of milk and three million metric tons of meat annually to world food supplies, much of it in parts of the world that are prone to nutritional imbalances. In addition they are a major source of draft power in much of the undeveloped world, which is why buffalo have been called the 'live tractors of the East' (Cockrill, 1970). Therefore, it is surprising that very little resources and scientific effort have gone into developing superior buffalo breeds similar to that of cattle, even though there is abundant genetic variety of this species. Sound breeding programs may also produce good quality milk.

Buffalo are the second largest source of milk supply in the world. In 2004, according to statistics from the United Nations' Food and Agriculture Organization (FAO), the world production of buffalo milk was 75.8 million metric tons. Trends in world milk production over the five years from 2000 to 2004 indicate that the volume of buffalo milk is increasing steadily at about 3% per year. While dairy cattle produce 84% of the total milk in the world it has to be noted that this volume is with an average fat and protein content of 4% and 3.5%, respectively (FAO, 2007). The average fat content in buffalo milk is about 7.5%

© Woodhead Publishing Limited, 2010

to almost 9%, while protein content in buffalo milk ranges from 4% to almost 4.5%. Thus, in terms of energy-corrected milk, buffalo milk accounts for about twice the food contribution suggested by the actual volume of buffalo milk produced yearly.

Buffalo are less tolerant of extremes of heat and cold than most cattle breeds. Their body temperature is lower than that of cattle in spite of the fact that their thick black skin (almost three times thicker than a cow hide) absorbs the sun's radiant heat energy very effectively. A buffalo's skin has only one-sixth the density of sweat glands as a cow. This is why buffalo like to wallow in water or mud pools when the temperature and humidity are high (Jainudeen, 2003). Regulation of body temperature in this way influences feed intake, reproduction and milk production. The limited application of AI programs for breed improvement and the overall lack of selective breeding at the village farm level has been the main bottleneck in the development of buffalo production (Nanda and Nakao, 2003). In general, a dairy cow heifer is considered to be efficient if the age at first calving is about 24–30 months. The calving interval should be about 12–13 months, with a lactation length of about 300 days, a 60–90 day dry period, and milk production of between 6000 and 7000 kg per lactation. Compared to buffalo with its own species-specific productive and reproductive traits, a buffalo heifer is on average around 40 to 60 months at first calving (Ganguli, 1981). However, there are indications that productive traits can be improved through selective breeding practices. As an example, Mediterranean breeds and swamp buffalo calved earlier than those from the Indian subcontinent (Rao and Nagarcenkar, 1977). The average calving intervals for Indian and Pakistani buffalo ranged from 15 to 18 months. The dry period has been reported to be 90 to 150 days for the Nili-Ravi breed of Pakistan, while for the Murrah it ranges from 60 to 200 days (Wahid, 1973). The average lactation length ranged from 252 to 270 days. As a result of these factors the productive life of a dairy buffalo is only 39% of its total life, compared to a stunning 52% in developed modern dairy cattle breeds (Ganguli, 1981; Sastry *et al.*, 1988).

IP Address: 129.132.208.100

In most of the buffalo milk-producing countries of Asia and the Indian subcontinent, it is observed that there are large seasonal variations in breeding and calving in buffalo (Ganguli, 1981). In India and Pakistan, 80% of the buffalo calve during June and December, causing a decline in milk production from March to June. Production starts increasing in June, to peak around September–October before declining again. However, this early summer decline in milk production could be due to heat stress and shortage of high quality forage greens. A dark body, lesser density of sweat glands and thick epidermis make it difficult for buffalo to thrive in extremely hot and dry conditions and these are also the months when the working draft buffalo are in the fields, plowing and cultivating next season's crops. Buffalo have developed survival mechanisms to seek water for immersion in these conditions, but extreme heat and cold significantly affect their milk production and reproductive efficiency (Sastry *et al.*, 1988). In addition to climatic influences, it is clear that poor nutrition and management have an adverse effect on breeding and milk production.

© Woodhead Publishing Limited, 2010

Changes in breeding, feeding and management can bring about notable improvements in the milk production and reproductive performance of buffalo (Sastry *et al.*, 1988). An obvious though neglected target has been finding out why the first calving occurs at such a late age. Close attention from birth to the time heifers reach breedable body size could bring down the age at first calving by six to nine months (Sastry and Tripathi, 1988). It has been shown that balanced feeding could bring buffalo heifers into cycle when they reach 330 kg body weight. There are also cases where heifers have calved at 20 to 24 months (Ganguli, 1981). Calving intervals in buffalo are influenced by the irregular and silent heat period as well as some reported irregularities in reproductive hormones throughout the year. It has been reported that there is seasonal breeding in buffalo due to diminished sexual activity in the period between March and June (Ganguli, 1981). Although buffalo are thought to be seasonal breeders, it has also been reported that they can breed throughout the year if reproduction management is good (Rao and Nagarcenkar, 1977; Sastry and Tripathi, 1988). Thus, it seems clear that there are several important management factors to consider in order to improve milk production in buffalo. These include managing the nutritional status of the dam around calving, pre- and post-partum hygiene, good milking management, balanced feeding throughout the year, oestrus detection and artificial insemination, managing thermal stress and improving housing – all good animal husbandry practices.

IP Address: 129.132.208.100

## 14.8 References

ADDEO, F., MERCIER, J.C. and RIBADEAU-DUMAS, B. (1977), The caseins of buffalo milk. *Journal of Dairy Research*, **44**, 455–468.

ALIEV, M. G. (1969), Physiology of milk ejection in buffaloes. *Dairy Science Abstracts*, **31**(12), 677–680.

ALIEV, M.G. (1970), Physiology of machine milking in buffaloes. *Dairy Science Abstracts*, **32**(3), 329–333.

ALIM, K.A. (1982), Aspects of milking technique and productivity of udder quarters in buffalo. *World Review of Animal Production*, **18**, 33–41.

BADRAN, A.E. (1992), Effect of vacuum and pulsation rate on milking ability in Egyptian buffaloes. *Buffalo Journal*, **1**, 1–7.

CADY, R.A., SHAH, S.K., SCHERMERHORN, E.C. and MCDOWELL, R.E. (1983), Factors affecting performance of Nili-Ravi buffaloes in Pakistan. *Journal of Dairy Science*, **66**, 578–586.

CHANTALAKHANA, A.T. (1999), Research priorities for small holder dairying. In: *Small Holder Dairying in the Tropics*, eds L. Falvey and C Chantalakhana, International Livestock Research Institute, Kenya, p. 403.

COCKRILL, W.R. (1970), The water buffalo. *Science Journal*, **6**(2), 35–40.

COCKRILL, W.R. (1974), Management conservation and use. In: *The Husbandry and Health of the Domestic Buffalo*, ed. W.R. Cockrill, Food and Agriculture Organization of the United Nations, Rome, pp. 276–312.

CZULAK, J. (1964), Manufacture of Gouda and Cheddar type cheeses from buffaloes milk.

© Woodhead Publishing Limited, 2010

*Australian Journal of Dairy Technology*, **19**, 166.

CZULAK, J., CONOCHIE, J., SUTHERLAND, B.J. and VAN LEEUWEN. H.J.M. (1969), Lactose, lactic acid, and mineral equilibria in Cheddar cheese manufacture. *Journal of Dairy Reseearch*, **36**, 93–101.

FALVEY, L. (1999), The future for small holder dairying. In: *Small Holder Dairying in the Tropics*, eds. L Falvey and C. Chantalakhana, International Livestock Research Institute, Kenya, p. 417.

FAO (2007), Food *Outlook – Global Market Analysis, Milk and Milk Products*, Food and Agriculture Organization of the United Nations, Rome.

GANGULI, N.C. (1981), Buffalo as a candidate for milk production. *International Dairy Federation Bulletin*, **137**.

GUO, M.R. (1993), *Goat's Milk and Goat's Milk Product Processing*, Heilongjiang Science and Technology Press, Harbin, China.

GUO, M.R. (2003), Changes in chemical composition of buffalo milk during 2003 (unpublished data).

HOFI, A.A., HAMZAWI, L.F., MAHRAN, G.A. and ASKER, A.A. (1977), Studies on buffalo milk fat globule membrane. I. Effect on stage of lactation. *Egyptian Journal of Dairy Science*, **5**, 235–240.

HÖGBERG, S.M. (2004), *Buffalo Milk Production*, Food and Agriculture Organization of the United Nations, Rome.

JAINUDEEN, M.R. (2003), Buffalo husbandry / Asia. In: *Encyclopedia of Dairy Science*, ed. H. Roginski, Academic Press, Amsterdam, pp.187–193.

KAY, H.D. (1974), Milk and milk production. In: *The Husbandry and Health of the Domestic Buffalo*, ed. W.R. Cockrill, Food and Agriculture Organization of the United Nations, Rome, pp. 329–376.

LALL, D. and NARAYANAN, K.M. (1991), Effect of lactation number on yield of milk solids-not-fat in different breeds of cows and Murrah buffaloes. *Indian Journal of Animal Science*, **61**, 433–435.

MASON, I.L. (1974), Environmental physiology. In: *The Husbandry and Health of the Domestic Buffalo*, ed. W.R. Cockrill, Food and Agriculture Organization of the United Nations, Rome, pp. 301–328.

NANDA, A.S. and NAKAO, T. (2003), Role of buffalo in the socioeconomic development of rural Asia: Current status and future prospectus. Animal Science Journal, **74**, 443–455.

NATIONAL RESEARCH COUNCIL (1988), *Nutrient Requirements of Dairy Cattle*, 6th edn, National Academy Press, Washington, DC.

PATEL, R.S. and MISTRY, V.V. (1997), Physicochemical and structural properties of ultrafiltered buffalo milk and milk powder. *Journal of Dairy Science*, **80**, 812–817.

RAO, M.K. and NAGARCENKAR, R. (1977), Potentialities of the buffalo. *World Review of Animal Production*, **13**, 53–62.

SAHAI, D. (1996), *Buffalo Milk: Chemistry and Processing Technology*. SI Publications, Karnal, India.

SAINI, S.S., SHARMA, J.K. and KWATRA, M.S. (1994), Prevalence and etiology of subclinical mastitis among crossbred cows and buffaloes in Punjab. *Indian Journal of Dairy Science*, **47**, 103–106.

SASTRY, N.S.R. and TRIPATHI, V.N. (1988), Modern management innovations for optimizing buffalo production. Buffalo production and health, A compendium of latest research information based on Indian studies. *Proceedings of the 2nd World Buffalo Congress*, Indian Council of Agricultural Research, New Delhi, pp. 38–62.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

SASTRY, N.S.R., BHAGAT, S.S. and BHARADWAJ, A. (1988), Aspects to be considered in milking management of buffaloes. *Indian Journal of Animal Production and Management*, **4**, 378–393.

TAILOR, S.P. and JAIN, L.S. (1993), Seasonality in buffalo reproduction. *Livestock Adviser*, **18**, 5–9.

TOLL, G.L. and HALNAN, R.E. (1976), The Giemsa banding pattern of the Australian Swamp buffalo (*Bubalus bubalis*): Chromosome homology with other Bovidae. *Canadian Journal of Genetics and Cytology*, **18,** 303–310.

WAHID, A. (1973), Pakistani buffaloes. *World Animal Review*, **7**, 22–28.

WHYTE, R.O. and MATHUR, M.L. (1965a), An analysis of the feed and fodder resources for livestock population of India. *Indian Dairyman*, **17**, 323–333.

WHYTE, R.O. and MATHUR, M.L. (1965b), The concentrate feed situation for dairy and poultry industries in India. *Indian Dairyman*, **17**, 233.

WILLIAMSON, G. and PAYNE, W.J.A. (1965), *An Introduction to Animal Husbandry in the Tropics*, 2nd edn. Longmans Green, London.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

# 15

# Milk quality requirements for yoghurt-making

**R. K. Robinson, formerly of the University of Reading, UK and M. S. Y. Haddadin, University of Jordan, Jordan**

**Abstract:** The sensory properties of natural yoghurt depend upon the chemical composition of the base milk, the method of processing and the characteristics of the starter culture. It is the importance of the former factors that is discussed in this chapter. The essential roles of milk proteins in coagulum formation are considered with special reference to bovine milk, but the relevance of seasonal variations in fat, minerals and vitamins with respect to product quality are noted, as are the impacts of heat-treatment and homogenisation.

**Key words:** yoghurt, composition of bovine milk, sensory properties.

IP Address: 129.132.208.100

## 15.1 Introduction

For hundreds, if not thousands, of years, rural communities in the Middle East turned the surplus milk from their herds of sheep or goats into various types of fermented milk. The traditional process involved pouring the milk into a large cooking pot, and reducing its volume by heating the milk over an open fire. After cooling the milk to roughly blood temperature, the milk was divided between a number of animal skin bags or earthenware pots which had held previous batches of product. Slowly, the residual microflora in the vessels attained sufficient activity to ferment the fresh milk into a yoghurt-like product that was, in nutritional terms, a most welcome addition to the diet. In addition, the low pH of the product tended to prevent putrefaction of the valuable milk proteins by spoilage bacteria, even at the ambient temperatures encountered in

© Woodhead Publishing Limited, 2010

the Gulf Region; it is likely also that the sensory properties of the products were preferred to those of the original milk (Tamime and Robinson, 2007).

The modern version of this traditional natural yoghurt with its sharp, acidic taste still remains popular in many parts of the world, and is usually consumed in a 'gel' form referred to as 'set yoghurt'. However, the major demand is now for the sweetened, semi-fluid product, 'stirred yoghurt', into which manufacturers have introduced a wide range of fruit flavours.

Under the current legal or non-legal regulations applied in most countries, both set and stirred yoghurts have to be fermented with a bacterial culture, and the traditional yoghurt culture consists of roughly equal numbers of two species, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*. Viable counts of each species in excess of $1.0 \times 10^6$ colony-forming units (cfu) $ml^{-1}$ of retail product are normal, and the presence of these 'live' bacteria has given rise to much speculation that yoghurt has 'health-promoting' properties beyond those associated with the base milk (Sellars, 1991). As with many issues linked with human nutrition, this controversy is difficult to resolve, and the same problem applies to the alleged prophylactic or therapeutic benefits of consuming yoghurts fermented with one of the so-called 'probiotic' cultures. These cultures consist of species like *Lactobacillus acidophilus* and *Bifidobacterium infantis* that are amongst the usual microbial inhabitants of the human intestine, and hence it is suggested that their ingestion in a yoghurt could be beneficial. Although classified as 'lactic acid bacteria' these intestinal species are poorly adapted to growing in milk, and commercial cultures of probiotic bacteria usually include *Strep. thermophilus* to ensure that a reasonable fermentation time is achieved.

Whatever the 'health-promoting' value of cultures incorporating *L. acidophilus*, *Bif. infantis* and *Strep. thermophilus* may be, it is not disputed that the same organisms secrete lower levels of lactic acid than a traditional yoghurt culture of *Strep. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*. As a result, the retail yoghurts have a mild taste that appeals to many consumers (Robinson, 2000a), and this factor alone may explain the rapid market expansion of probiotic yoghurts. Incidentally, current legislation in the European Union requires that all these set or stirred products should, irrespective of the composition of the starter culture, be designated as 'yoghurt' (Hickey, 2005), and this proposal appears to have found universal acceptance.

The introduction of fruit yoghurts during the 1950s and, more recently, the probiotic yoghurts were developments that were essential to bring yoghurt to a wider market. Nevertheless, what is interesting about the yoghurt-making process is that, in essence, the procedure employed today to manufacture the millions of gallons of yoghurt that are sold each year (IDF, 2005) has changed little from that discovered by the desert nomads many centuries ago. Thus, a typical modern process consists of the following stages, in which homogenisation is the only step that does not have a traditional equivalent:

1. Standardisation of fat and protein contents of the milk.
2. Homogenisation at 15–20 MPa and 50–55ºC.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

3. Heat treatment of the standardised milk at 80–85ºC for 30 min or 90–95ºC for 5–10 min.
4. Cooling to 30 or 42ºC and inoculation with a culture.
5. Filling into cartons for set yoghurt or holding in incubation tank for stirred yoghurt.
6. Incubation for 16 hours at 27–30ºC or 4–5 hours at 42ºC.
7. Cooling to 2–4ºC for set yoghurt in cartons, or 20–25ºC for stirred yoghurt in tanks.
8. Addition of fruit purée or fruit flavours to yoghurt base of stirred varieties, packaging, and cooling of sealed cartons to 2–4ºC.
9. Retail products – natural set yoghurt and stirred fruit yoghurt are usually sold in 120–150 g individual cartons or 500 g family packs (Robinson, 2000b).

Today, flavouring and sweetening agents often dominate the sensory properties of a retail yoghurt, but the character of a good quality, traditional yoghurt has many facets. It should offer a background flavour note derived from the presence of acetaldehyde, a fresh, acidic taste imparted by the lactic acid, alongside the smooth, attractive 'mouthfeel' of the soft coagulum. The generation of these essential properties is dependent on the correct manipulation of the components of raw milk, and the method(s) of achieving this aim are discussed below.

IP Address: 129.132.208.100

## 15.2 Base milk for yoghurt-making

### 15.2.1 Essential components from bovine milk

Fresh bovine milk is the usual raw material for making yoghurt in factories across the world, and the critical feature of this milk is the level of solids-non-fat (SNF) which, in bovine milk, varies from 85 to 90 $g\,l^{-1}$. More specifically, the quality of yoghurt depends upon the protein content of the process milk, and around 33 $g\,l^{-1}$ (26 g casein and 7.0 g whey proteins) is present in the raw milk. Lactose at around 45 $g\,l^{-1}$ forms the bulk of the SNF with the balance being minerals. The role of lactose is to provide a substrate for the fermentation stage, while minerals like calcium and phosphorus, along with the proteins, give rise to the basic yoghurt coagulum. The fat content of bovine milk, depending on breed of cow and diet, tends to be in the region of 30–35 $g\,l^{-1}$ but, despite the high level, it plays no part in the formation of the yoghurt gel. It is important with respect to quality as perceived by the consumer, but a value of 10–12 $g\,l^{-1}$ is more than adequate to provide yoghurt with a smooth, satisfying 'mouthfeel'. Consequently, the fat content of the raw milk has to be reduced to a preset level by centrifugal separation before the bulk milk can be further processed.

However, although milk may be the ideal food for infants, the levels of protein present in liquid milk are not sufficient to produce an attractive yoghurt in terms of consistency (set yoghurt) or viscosity (stirred yoghurt). Unmodified

© Woodhead Publishing Limited, 2010

bovine milk can be coagulated to form a soft gel suitable for use as an ingredient in cooking, but few people would eat the product for pleasure. As mentioned earlier, this problem was recognised long ago, and hence the first step in yoghurt-making was always to raise the SNF content by heating the fresh milk in an open pan suspended over a fire (Tamime and Robinson, 2007). The aim of this action was to raise the total solids in the milk by removing some of the water, and today a similar end-point is achieved by vacuum evaporation or ultra-filtration.

### 15.2.2 Standardisation

What average figures for the fat or protein contents of bovine milk disguise is variation, for differences of up to 6.5 g $l^{-1}$ of protein can be observed between breeds of cow (Tamime and Robinson, 2007), and 4–5 g $l^{-1}$ for a specific breed of cow across the seasons. Therefore, before the bulk milk can be standardised prior to processing, the manufacturer needs to know two things: (a) the gross chemical composition of the milk in the storage silos, and (b) the protein and fat contents desired in the final product. Taking a representative sample of the bulk milk (IDF, 1985) will enable the laboratory to obtain the compositional figures within a few minutes using infrared systems of analysis (Anderson *et al.*, 1993; Bintsis *et al.*, 2008), while the product values should be available in-house. Thus, by means of informal taste panels and instrumental measurements, every manufacturer should have a quality profile of every product line within the factory, and the values of protein and fat required in the yoghurt base(s) to achieve these profiles can be easily established. Alternatively, reference may be made to the levels of SNF being sought, so that a typical base for a stirred fruit yoghurt might contain ~140 g $l^{-1}$ non-fat milk solids, as against 85–90 g $l^{-1}$ in the bulk raw milk; higher levels of SNF might be sought in 'luxury' or Greek-style yoghurts (Robinson, 2000a).

In large plants, this elevation of the SNF is usually achieved by evaporation under vacuum (EV) or ultra-filtration (UF) (Tamime *et al.*, 1984). Either process can remove water and raise the levels of both fat and protein in the yoghurt base, but some lactose will be lost during the UF process (Robinson *et al.*, 2002); a loss of minerals through a UF membrane would be expected as well, but the published results are inconsistent, perhaps due to the use of different membranes (Tamime and Robinson, 2007). In terms of product quality, the loss of soluble material appears unimportant, and certainly the level of lactose in UF milk (see Table 15.1) is ample to support the growth of a starter culture of lactic acid bacteria. Some authors have shown that milk standardised by UF treatment gives rise to a yoghurt with, as measured instrumentally, improved gel strength compared with yoghurts made from EV milk or milk fortified with skim-milk powder (Abrahamsen and Holmen, 1980). However, Abrahamsen and Holmen (1980) and Lankes *et al.* (1998) suggested that EV gives rise to a smoother coagulum that is preferred by consumers. This improvement in sensory properties may result from the fact that the milk is heated more severely during the EV

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

**Table 15.1** Chemical composition of yoghurt milks (g $l^{-1}$) concentrated by different methods. Note that few, if any, minerals appear to have been lost during the UF process

| Treatment | Total solids | Fat | Protein | Lactose | Ash |
|---|---|---|---|---|---|
| Original milk | 118.4 | 34.3 | 31.2 | 44.5 | 8.4 |
| Evaporation | 145.7 | 34.9 | 41.2 | 60.3 | 9.3 |
| Ultra-filtration | 141.3 | 36.0 | 49.7 | 46.3 | 9.3 |
| Skim-milk powder addition | 143.2 | 33.2 | 41.4 | 59.3 | 9.3 |

Source: after Abrahamsen and Holmen (1980).

process (perhaps 70–90ºC) (Bylund, 1995), as compared with 50–55ºC during UF processing. Exactly how these different temperature profiles affect the milk proteins and/or other constituents was not established. Concentration by EV or UF increases the fat content as well, and the initial cream separation step must be adjusted to allow for this elevation.

Irrespective of the process employed, the aim is to raise the level of protein in the milk to 40–50 g $l^{-1}$, with the precise value being selected in relation to the economics of the process and the physical properties desired in the end-product. In general, the higher the protein content of the milk, the stronger will be the resultant yoghurt gel, but this improvement does have a financial cost. Alternatively, the target value can be achieved through the addition of a milk-derived powder. Skim-milk powder is the obvious choice, but less expensive sources of milk protein may be used as well. Whey protein powders can increase gel firmness, so long as the casein:whey protein ratio in the process milk is controlled and the heat treatment of the mix is sufficient to denature the whey proteins and cause them to associate with the casein micelles (Puvanenthiran *et al.*, 2002; Antunes *et al.*, 2004). This latter requirement applies, of course, to all milks to be transformed into yoghurt and hence, whatever the procedure of standardisation, the process milk has to undergo a specified heat-treatment.

IP Address: 129.132.208.100

Although the fat content of a typical process milk may be around only 10 g $l^{-1}$, this is sufficient for a cream layer to be noticeable in natural set yoghurt, so homogenisation often precedes heat treatment. For milks with fat contents of >5 g $l^{-1}$ homogenisation becomes optional, even though it may be included in a process line to dissolve thoroughly dry ingredients like skim-milk powder.

### 15.2.3 Effect of homogenisation

Once the desired composition of milk in terms of fat and SNF has been achieved, the milk will usually be homogenised using pressures of 15–20 MPa at 55–70ºC. The advantage of homogenisation is the reduction in size of naturally occurring fat globules with diameters up to 10 $\mu$m down to globules with a uniform size of <2 $\mu$m. This removal of the large globules avoids the risk of their coalescence and the formation of a visible fat layer; the apparent whiteness of natural yoghurt is enhanced as well. Furthermore, the viscosity of the yoghurt

© Woodhead Publishing Limited, 2010

base may be enhanced by homogenisation, in that a portion of the casein and whey proteins becomes attached to the surfaces of the newly formed fat globules, so effectively binding the fat into the structure of the yoghurt coagulum (Walstra, 1998); native fat globule membranes do not interact with proteins in the same way (van Vliet and Dentener-Kikkert, 1982). However, due to the disruption of the natural fat globule membranes and the release of lipase, homogenised milks should be heat treated without delay in order to reduce the risk of rancid off-flavours developing.

In many factories, homogenisation precedes the heat treatment stage on the grounds that, even after cleaning, the complex mechanism of the high pressure head might still harbour contaminant bacteria and yeasts. The use of cleaning-in-place systems has tended to reduce this risk and, given this extra security, Walstra (1998) has advocated that homogenisation should follow the heat treatment step. Thus, the physical properties of the finished yoghurt are enhanced by whey protein/casein interactions induced during heat treatment of the process milk, and Walstra (1998) suggested that these interactions could be facilitated by homogenisation of the previously heated milk. The validity or otherwise of this idea has yet to be established during full-scale operations, and many operators prefer to retain their existing layout and to heat-process the milk just prior to the fermentation step.

### 15.2.4 Effect of heat treatment

IP Address: 129.132.208.100

Heat treatment is an essential stage in yoghurt-making, and two alternative systems are widely practised. The High Temperature–Short Time (HTST) system involves passing the milk through a plate heat exchanger to raise the temperature to 90–95ºC, and then holding the milk at that temperature for 5–10 minutes before cooling. In contrast, the Low Temperature Holding (LTH) system has the milk heated in a process vessel to 80–85ºC for 30 minutes. The HTST system is the most convenient for large-scale operations handling thousands of gallons of milk per week, but a number of workers have shown experimentally that the LTH approach gives a yoghurt with better firmness and viscosity than yoghurt made with HTST-processed milk; however, the difference is not sufficiently great as to negate the advantages of the HTST process (Tamime and Robinson, 2007).

What is important, though, is that the heating should be at a temperature above 80ºC and over an extended period of time, and it is this latter demand that makes it impossible to produce a good quality natural yoghurt from ultra-high temperature (UHT) treated milk. Thus, the extended holding period allows time for the physico-chemical properties of the caseins to be altered, and the whey proteins to be denatured; >80% of the $\beta$-lactoglobulin is affected by a typical LTH treatment. This denatured $\beta$-lactoglobulin then becomes attached to the $\kappa$-casein and, when viewed using transmission electron microscopy, the whey proteins appear as fine appendages to the casein micelles (Harwalkar and Kalab, 1986). During the fermentation stage, these fine strands of whey protein prevent

© Woodhead Publishing Limited, 2010

the casein micelles from forming the firm aggregates associated with cheese curd, and it is this restricted aggregation that gives rise to the soft coagulum desired in the final product (Modler and Kalab, 1983; Mottar *et al.*, 1989). In addition, whey protein denaturation leads to coagulation of the milk at a higher pH (see later) than would be normal with non-heated milk, so reducing process times and leading to improved crosslinking between the micelles (Lucey and Singh, 1997). This increased number of bonds between the strands of protein increases the rigidity of the casein network and its apparent water-holding capacity (Ozer *et al.*, 1998; Lee and Lucey, 2003).

It is this impact of heating/holding on the physical properties of yoghurt that makes the stage unavoidable, but the same procedure does have other actions on the process milk that are reflected in the quality of the retail product. In particular, the heating will cause a release of free amino acids from the denatured whey proteins, amino acids that may stimulate the metabolism of the starter culture with respect to the synthesis of flavour-enhancing compounds. For example, both threonine and methionine can act as substrates for the formation of acetaldehyde by *L. delbrueckii* ssp. *bulgaricus* and *Strep. thermophilus* and, as this metabolite is the principal component of the typical flavour of natural yoghurt, enhancing its production is clearly beneficial (Lees and Jago, 1978). In addition, heating the milk causes (a) an expulsion of oxygen from the milk, so encouraging the growth of the micro-aerophilic starter bacteria; (b) the eradication of any non-spore-forming pathogens like *Salmonella* spp. or *Escherichia coli* O157 that might have been present in the raw milk; and (c) a reduction in the overall bacterial or fungal microflora in the process milk that might otherwise compete against the starter culture, e.g. *Pseudomonas* spp., and/or cause spoilage of the packaged yoghurt during transport or in-store display, e.g. *Saccharomyces* spp. or *Aspergillus* spp.

IP Address: 129.132.208.100

## 15.3 Establishing the conditions for coagulation

After the heat-treatment stage, the milk will be cooled via a plate heat exchanger to 42–43ºC, or 27–30ºC for overnight fermentation, and pumped either to an insulated fermentation vessel for inoculation with a starter culture and incubation (stirred yoghurt) or to a holding tank for inoculation and direct packaging into retail cartons prior to incubation; small dairies may carry out the inoculation and/or fermentation in the vessel previously employed to heat-treat the milk with the LHT system. The method of inoculation and the rate of addition of the starter culture depend upon both the physical form of the culture, i.e. liquid, concentrated freeze-dried or concentrated frozen, and the viable cell count(s) per gram or millilitre of culture as purchased from a commercial supplier; a number of systems of inoculation have been developed to avoid contamination of the milk with undesirable airborne bacteria or fungi (Tamime, 2002).

As mentioned earlier, the traditional yoghurt culture consists of strains of *L. delbrueckii* ssp. *bulgaricus* and *Strep. thermophilus* but, today, many yoghurts

© Woodhead Publishing Limited, 2010

are manufactured with 'probiotic' cultures including *Lactobacillus* spp. and *Bifidobacterium* spp. of human intestinal origin, along with *Strep. thermophilus* for the rapid release of lactic acid. The choice of species/strains of each species, the ratio between the species, the rate of inoculation and the temperature of incubation can all affect the quality of the finished yoghurt in terms of flavour and texture but, as this text is focused on the impact of milk quality on yoghurt, the role(s) of the starter culture will not be explored in detail.

Once the cartons for set yoghurt have been filled with the inoculated milk, they are sealed and placed into holding trays in an incubation room at 42ºC or, alternatively, the trays are transferred to a conveyor belt that slowly runs through a tunnel operated at the same temperature. By contrast, the base for stirred yoghurt is fermented in bulk, and the important features of a typical fermentation tank are good temperature control during incubation, and a method for stirring/cooling the bulk yoghurt once the preset pH has been reached. Numerous variations of these two systems can be found across the dairy industry, but the essential demand on all of them is that the inoculated milk should be given time – usually 4–5 hours – to form a gel. Gelation will also occur at 30ºC over a period of around 16 hours, but the use of this temperature tends to find favour only with small-scale operators.

## 15.4 Formation and structure of yoghurt coagulum

IP Address: 129.132.208.100

The activity of a traditional starter culture will generate around 10–12 $g\,l^{-1}$ lactic acid (around pH 4.2–4.3) after 3–4 hours but, with a culture in which probiotic species dominate, a final pH of 4.5–4.6 may take 4–5 hours to achieve. This prolonged incubation time is a reflection of the inability of species like *L. acidophilus* and *Bif. infantis* to generate lactic acid as rapidly as species better adapted to milk. However, as the survival of the probiotic bacteria over the shelf-life of the product is reduced at low pH, a pH of 4.5–4.6 is a good target figure. In addition, as long as the pH is below the isoelectric point of casein-whey protein particles formed during the heat treatment of the milk (pH ~ 5.3), the milk proteins will coagulate to form a gel (Lucey and Singh, 1997, 2003).

Thus, casein micelles in bovine milk at pH 6.7 have a net negative charge which keeps them suspended as discrete entities, but the build-up of lactic acid results in the surface charge (zeta potential) on each micelle falling to almost zero. This absence in surface charge eliminates the repulsion between micelles, and allows them to aggregate at the isoelectric point of native casein (pH ~ 4.6) through hydrophobic and electrostatic bonds (Robinson *et al.*, 2006). However, in milk heated above 80ºC and held for an appropriate period of time, one of the main whey proteins, namely $\beta$-lactoglobulin, is denatured and becomes bonded to the casein micelles. Initial aggregation of the casein-whey protein particles occurs, therefore, around the isoelectric point (pH ~ 5.3) of the $\beta$-lactoglobulin (Lucey and Singh, 2003).

© Woodhead Publishing Limited, 2010

The role of colloidal calcium phosphate (CCP) in maintaining the stability of casein micelles is also important (Horne, 1999), especially as the CCP gradually dissolves during acidification (Lucey, 2002); by pH 5.1, most of the CCP has solubilised. As the gelation point of the casein-whey protein particles is around pH 5.3, some CCP continues to be dispersed even after the initial network of casein particles has formed. Initially, this loss of CCP causes a 'loosening' of the network and a softening of the gel, a 'fault' that is reversed as the pH of the yoghurt falls below pH 5.0; gel firmness reaches a maximum at pH ~4.6 – the isoelectric pH of casein – and may further increase with time as more protein-protein bonds are established (Ozer *et al.*, 1998).

Although 42ºC is the usual fermentation temperature for traditional yoghurt, the use of lower temperatures with 'probiotic' cultures, e.g. 38ºC, and slightly longer incubation times can give rise to firmer gels that are less prone to whey separation; this is important for natural yoghurt coagulated in the carton (Lucey, 2002; Lee and Lucey, 2003). By contrast, incubation temperatures above 42ºC make the bonds within the protein network prone to cycles of breakage and reformation, and these changes can lead to excessive syneresis (Lucey, 2001; Mellema *et al.*, 2002). Elevated incubation temperatures can also encourage the formation of 'lumps' in bulk stirred yoghurt (Robinson, 1981), but the insertion of strainers in the process line or high-speed mixing of the finished product means that any 'lumps' can be disintegrated mechanically ahead of filling.

### 15.4.1 Coagulation of other milks

IP Address: 129.132.208.100

Although most of the yoghurt sold in Europe, North America and Australasia is manufactured from bovine milk, the climate and terrain of countries around the Mediterranean and across the Middle East make camels, sheep and goats the domestic herbivores of first choice. Not surprisingly, the average chemical composition of the three milks differs, both quantitatively and qualitatively, from bovine milk but, even so, they have been widely used as the bases for various types of fermented product.

For example, camel milk contains ~45 $g\,l^{-1}$ lactose that can be fermented readily by mesophilic or thermophilic cultures to give an acceptable, refreshing drink but, even at the anticipated isoelectric point of the caseins (pH 4.6), gelation of the milk does not occur (Attia *et al.*, 2001; Haddadin *et al.*, 2008). The reason for this absence of coagulation is not clear, but Ali and Robinson (1985) suggested that the size of the micelles is too small to allow for the formation of the dense protein network observed in yoghurt made from bovine milk. A soft gel suitable for cheesemaking can be produced (Haddadin *et al.*, 2007), but pressing is needed to give the product the desired texture.

Even though the other animal popular in semi-arid regions, the sheep, has a lactation period of only about six months, ovine milk does make an excellent yoghurt in terms of flavour and texture/viscosity (Anifantakis, 1990; Tamime *et al.*, 1993); with a typical protein content of 56 $g\,l^{-1}$, there is no need to fortify the base milk. Lowering of the milk fat content may be undertaken for some

© Woodhead Publishing Limited, 2010

urban markets – ovine milk from some breeds may contain $75\,g\,l^{-1}$ – but otherwise, good quality yoghurt can be manufactured by employing the process already outlined for bovine milk.

Caprine milk is sometimes recommended for infants who are allergic to the proteins in bovine milk, but the consistency or viscosity of yoghurts made from caprine milk standardised by EV or UF can be something of a disappointment when compared with products of bovine or ovine origin. Tamime and Marshall (1997) reported that, in comparison with bovine and ovine milks, caprine milk had the lowest level of $\alpha$-casein and the highest level of $\beta$-casein, but whether or not this change in the balance between the caseins is solely responsible for the weak gelation properties of caprine milk was not discussed. Vlahopoulou *et al.* (1994) suggested that gel firmness could be improved by using a low inoculation rate, namely $<15\,ml\,l^{-1}$, of a liquid yoghurt culture containing *L. delbrueckii* ssp. *bulgaricus* and *Strep. thermophilus*, and it may be that the slow release of lactic acid has an effect equivalent to the incubation of bovine milk at temperatures below 42º. The intrusion of a 'goaty' flavour associated with free short-chain fatty acids and low concentrations of acetaldehyde means that natural caprine yoghurt can lack the attractive flavour of the bovine or ovine products (Abrahamsen and Rysstad, 1991). Stelios and Emmanuel (2004) suggested that it would be better to use caprine milk to supplement the limited volumes of ovine milk available in Greece and elsewhere, for mixtures of caprine:ovine milk in the ratios of 50:50 or 70:30 gave better quality yoghurts (with respect to both rheological and sensory properties) than caprine milk alone. Such an approach could also make sense economically, as ovine milk tends to be expensive.

IP Address: 129.132.208.100

## 15.5 Factors that affect coagulation

### 15.5.1 Extraneous materials in milk

The naturally occurring antimicrobial systems in bovine milk, e.g. the lactoperoxidase system, are alleged to be important in protecting the calf from diseases of bacterial origin but, as the active agents are heat-labile, the heat treatment of the yoghurt milk is sufficient to ensure that they do not adversely affect the starter culture.

However, while milk may help the calf to avoid infections, the udder from which it is drawn is highly susceptible to one important disease, bacterial mastitis (Chambers, 2002). Treatment of this disease involves the intra-mammary injection of antibiotics, such as penicillin or streptomycin, which, over a period of a few days, pass from the tissues of the udder into the milk. In many countries, farmers are required to withhold the milk from treated animals from the market for three days, a practice that can represent a considerable economic loss to the farmer. Consequently, there will be occasions when the milk from certain cows is not held back as long as it should be, and the bulk milk leaving the farm will contain residues of an antibiotic. From a public health

© Woodhead Publishing Limited, 2010

standpoint, traces of antibiotics in foods are highly undesirable but, for the yoghurt-maker, their presence can result in a major vat failure. Thus, *Strep. thermophilus*, which is found in most thermophilic starter cultures, is extremely sensitive to the widely used antibiotics, and levels of penicillin as low as 0.004 International Units (IU) can inhibit growth. Even in the presence of the more resistant *L. delbrueckii* ssp. *bulgaricus*, 0.01 IU of penicillin can adversely affect the yoghurt fermentation, and a number of other antibiotics can have a similar impact (Tamime and Robinson, 2007). The mixing of milk from a number of farms can have the benefit of diluting any antibiotic residues, but the impact of antibiotics on culture activity can be so severe that most factories will test all incoming milk. This approach has become possible because the current range of test kits can detect the commonly-used antibiotics in a matter of minutes (Bintsis *et al.*, 2008), so that security does not need to delay the production schedule.

Milks with somatic cell counts above $2.5 \times 10^5$ cells $ml^{-1}$ have been reported to give rise to yoghurts with impaired sensory properties (Rogers and Mitchell, 1994), but the impact seems to be marginal. Similarly, residues of the cleaning or disinfecting agents used to clean milking machines or process plant should not pose a problem, as mixed cultures of *L. delbrueckii* ssp. *bulgaricus* and *Strep. thermophilus* can often withstand the presence of $>100\,mg\,l^{-1}$ of chlorine- or iodine-based agents (Tamime and Deeth, 1980). However, *Strep. thermophilus* alone tends to be much more sensitive, so that a faulty cleaning system could pose problems if 'probiotic' cultures are being employed to make the yoghurt.

IP Address: 129.132.208.100

### 15.5.2 Natural changes in milk quality

The causes and nature of seasonal variations in the chemical composition of bovine milk are discussed elsewhere in this book and, as mentioned earlier, changes in the protein values are of special relevance to yoghurt-makers; the relationship between the protein content of the milk and the consistency of the final yoghurt is well established.

What are less frequently considered are seasonal changes in the vitamin and mineral concentrations in bovine milk (Robinson and Wilbey, 1998), and yet both components could affect the quality of yoghurt. For example, many starter cultures for yoghurt require an external source of B-group vitamins, and hence the levels in the process milk could influence the metabolism of the culture with respect to the synthesis of flavour compounds; the rate of acid production could also be affected. The concentrations of minerals like calcium could again impact on yoghurt quality, and Robinson (1981) cited evidence to suggest that 'nodules' – small hard lumps of milk protein – were present in yoghurt mainly in the spring or autumn, but whether seasonal changes in the mineral content were responsible for the 'nodules' was not established.

Taints in natural yoghurts derived from the casual consumption of a meadow flora including buttercups, wild camomile or other weed species tend to arise only where the milk from one farm is used for manufacture, for the commingling

© Woodhead Publishing Limited, 2010

of milks from a number of sources usually provides ample dilution. Potential taints resulting from access to poor quality silage should similarly be lost in the bulking of supplies.

## 15.6 Final steps in the process

The essential precaution when cooling set yoghurt fermented in retail cartons is the avoidance of mechanical disturbance. Consequently, the usual procedures are to blast cold air over stationary trays of yoghurt or, if a conveyor belt takes the trays into a chilled section of the tunnel, to ensure that the movement of the belt is absolutely smooth. If these conditions are met, cooling the cartons of yoghurt to a storage temperature around 5ºC results in the gel becoming firmer due to additional hydrogen bonds or disulphide crosslinks arising between denatured whey proteins and $\kappa$-casein (Ozer *et al.*, 2002; Vasbinder *et al.*, 2003).

In-tank cooling of the base for stirred yoghurt requires the circulation of chilled water (2ºC) through the jacket/in-tank cooling system of the incubation vessel, or the pumping of the warm yoghurt (42ºC) through a plate or tubular cooler to reach a temperature of 20–25ºC. Various off-line or in-line systems are available for mixing the yoghurt base with commercially sterile fruits or fruit flavours (Tamime and Robinson, 2007), but it is important, in the present context, that typical fruit blends contain (a) sucrose at levels of around 300–350 g $kg^{-1}$, (b) colouring and flavouring agents to ensure consistency of colour and flavour, and (c) stabilisers to protect the structure of the fruit pieces and contribute to the viscosity of the retail yoghurt. The ratio of fruit to white base will depend upon the image desired for the end-product, but the perceived sensory properties of a stirred fruit yoghurt always depend on the quality of the base. The choice of starter culture is relevant in this latter context because, while the culture for a natural set yoghurt may be selected on account of the concentration of acetaldehyde that it secretes, stirred yoghurt is often manufactured with cultures that generate extracellular polysaccharides. These materials vary in their properties but, in general, they will improve the viscosity of the yoghurt and, hence, the sensation of quality as perceived by a consumer (Laws and Marshall, 2001; De Vuyst *et al.*, 2003).

Although yoghurt manufacturers adopt packaging that will look attractive to consumers, amongst the critical purposes of the carton are (a) prevention of chemical or microbiological contamination of the product; (b) avoidance of exposure of the product to oxygen, for example, the risk of oxidative deterioration of milk lipids; and (c) exclusion of light, e.g. from fluorescent lights in a display cabinet, that could cause the bleaching of fruit colours. Individual polypropylene cartons with heat-sealed, aluminium foil closures or 'snap-on' polypropylene lids can achieve these aims, as can the 'nests' of cartons – usually four – generated by a form–fill–seal machine that takes a roll of film, thermoforms the cartons, fills each carton with a specific flavour of yoghurt, and then seals them with a foil lid. For large-scale operations, the form–

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

fill–seal system is more economic, but both types of carton can be equally good at maintaining the sensory properties of the yoghurt. Faults such as excessive whey separation can arise following severe mechanical shaking in transit or in-store temperature abuse but, in general, the quality of well-made yoghurt should not deteriorate in the carton.

The in-carton production of gas by yeasts or the visible growth of a mould can occasionally spoil retail products, but the good news is that bacterial pathogens of concern to humans are rarely found in well-made yoghurt.

## 15.7 Future trends

Many people have an inherent vision of 'yoghurt' as a gelled, rather acidic product or, more usually, as a 'spoonable' fruit-flavoured dessert with a viscous, custard-like 'mouthfeel', and manufacturers are careful not to stray too far from these expectations. Drinking yoghurts are acceptable in warm climates, e.g. Ayran in Turkey, but Europeans tend to turn to liquid fermented milks for the alleged therapeutic properties of the culture and/or the desire for special ingredients like phytosterols.

Whether the addition of nutraceuticals, i.e. food additives whose nutritional importance has not been proven unequivocally, to yoghurt will become more widespread remains an open question but, in any event, the forms of any additives, such as omega-3 fatty acids or phytosterols, are likely to be chosen so that they do not change the physical or sensory properties of the basic yoghurt (Awaisheh *et al.*, 2005). The use of special bovine diets to raise the level of unsaturated fatty acids in milk has also been suggested as an alternative to their addition to a retail product. Fermentations with starter cultures that reveal specific, desirable characteristics after ingestion could also become more widely publicised, for some strains of *L. acidophilus*, for example, are extremely effective in lowering serum cholesterol (Abdulrahim *et al.*, 1997). What is evident, however, is that all these possible innovations are essentially 'cosmetic', and the basic nature of the yoghurt will not be affected.

One possible change in the processing of yoghurt that could have a dramatic impact on the yoghurt coagulum involves the addition of an enzyme, transglutaminase (EC 2.3.2.13), to the base milk along with the starter culture. The action of this enzyme is to enhance the crosslinking between the milk proteins, and the gel strength of a test yoghurt was five times greater than that of a control without the enzyme (Lorenzen and Neve, 2003). However, it is notable that the enzyme would be retained in the yoghurt until the time of consumption, and some regulatory authorities might find this 'contamination' of the retail product a matter of some concern.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

## 15.8 References and further reading

ABDULRAHIM S, HADDADIN M S Y, ODETALLAH N H and ROBINSON R K (1997), 'A proposed protocol for checking the suitability of *Lactobacillus acidophilus* for use during feeding trials with chickens', *Trop Sci*, 74, 16–20.

ABRAHAMSEN R K and HOLMEN T B (1980), 'Yoghurt from hyperfiltrated, ultrafiltrated and evaporated milk and from milk with added milk powder', *Milchwissenschaft*, 35, 399–402.

ABRAHAMSEN R K and RYSSTAD G (1991), 'Fermentation of goat's milk with yogurt starter bacteria', *Cult Dairy Prod J*, 26(3), 20–22.

ALI M Z and ROBINSON R K (1985), 'Size distribution of casein micelles in camels' milk', *J Dairy Res*, 52, 303–307.

ANDERSEN T, BREMS N, BØRGLUM M M, KOLD-CHRISTENSEN S, HANSEN E, JØRGENSEN J H and NYGAARD L (1993), 'Modern laboratory practice – Chemical analyses', in Robinson R K, *Modern Dairy Technology*, Volume 2, London, Elsevier Applied Science, 381–416.

ANIFANTAKIS E M (1990), 'Manufacture of sheep's milk products', *XXII Int Dairy Congr*, Vol 1, 420–432.

ANTUNES A E C, ANTUNES A J and CARDELLO H M A B (2004), 'Chemical, physical, microstructural and sensory properties of set fat-free yogurts stabilised with whey protein concentrate', *Milchwissenschaft*, 59, 161–165.

ATTIA H, KHEROUATOU N and DHOUB A (2001), 'Dromedary milk lactic acid fermentation: microbiological and rheological characteristics', *J Ind Micro Biotechnol*, 26, 263–270.

AWAISHEH S S, HADADDIN M S Y and ROBINSON R K (2005), 'Incorporation of nutraceuticals and probiotic bacteria into a fermented milk', *Int Dairy J*, 15, 1184–1190.

BINTSIS T, ANGELIDIS A S and PSONI L (2008), 'Modern laboratory practices – Analysis of dairy products', in Britz T J and Robinson R K, *Advanced Dairy Science and Technology*, Oxford, Blackwell Publishing, 183–261.

BYLUND G (1995), *Dairy Processing Handbook*, Lund, Sweden, Tetra Pak Processing Systems AB.

CHAMBERS J V (2002), 'Microbiology of raw milk', in Robinson R K, *Handbook of Dairy Microbiology*, New York, John Wiley & Sons, 39–90.

DE VUYST L, ZAMFIR M F, ADRIANY T, MARSHALL V, DEGEEST B and VANINGELGEM F (2003), 'Exopolysaccharide-producing *Streptococcus thermophilus* strains as functional starter cultures in the production of fermented milks', *Int Dairy J*, 8, 171–177.

HADDADIN M S Y, GAMMOH S I and ROBINSON R K (2007), 'Nabulsi next', *Dairy Ind Int*, July, 24–27.

HADDADIN M S Y, GAMMOH S I and ROBINSON R K (2008), 'Seasonal variations in the chemical composition of camel milk in Jordan', *J Dairy Res*, 75(1), 8–12.

HARWALKAR V R and KALAB M (1986), 'Relationship between microstructure and susceptibility to syneresis in yoghurt made from reconsistuted non-fat dry milk', *Food Microstruct*, 5, 287–294.

HICKEY M (2005), 'Current legislation of probiotic products', in Tamime A Y, *Probiotic Dairy Products*, Oxford, Blackwell Publishing, 73–97.

HORNE D S (1999),' Formation and structure of acidified milk gels', *Int Dairy J*, 9, 261–268.

IDF (1985), 'Methods of analysis of milk and milk products', *IDF Bulletin* No. 193, Brussels, International Dairy Federation.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

IDF (2005), *World Dairy Situation – 2004*, Document No. 399, Brussels, International Dairy Federation.

LANKES H, OZER B H and ROBINSON R K (1998), 'Effect of method of fortification on the texture of natural yoghurt', *Milchwissenschaft*, 53(9), 510–513.

LAWS A P and MARSHALL V M (2001), 'The relevance of exopolysaccharides to the rheological properties of milk fermented with ropy strains of lactic acid bacteria', *Int Dairy J*, 9, 709–721.

LEE W and LUCEY J A (2003), 'Rheological properties, whey separation and microstructure in set-style yoghurt: effects of heating temperature and gelation temperature', *J Text Stud*, 34, 515–536.

LEES G J and JAGO G R (1978), 'Role of acetaldehyde in metabolism: A review', *J Dairy Sci*, 61, 1216–1224.

LORENZEN P C and NEVE H (2003), in *Fermented Milk*, Special Issue No. 0301, Brussels, International Dairy Federation, 241–249.

LUCEY J A (2001), 'The relationship between rheological parameters and whey separation in milk gels', *Food Hydrocoll*, 15, 603–608.

LUCEY J A (2002), 'Formation and physical properties of milk protein gels', *J Dairy Sci*, 85, 281–294.

LUCEY J A and SINGH H (1997), 'Formation and physical properties of acid milk gels: a review', *Food Res Int*, 30, 529–542.

LUCEY J A and SINGH H (2003), 'Acid coagulation of milk', in Fox P F and McSweeney P H L, *Advanced Dairy Chemistry*, Vol. 2, Gaithersburg, MD, Aspen Publishers, 1001–1026.

MELLEMA M, WALSTRA P, VAN OPHEUSDEN J H J and VAN VLIET T (2002), 'Effects of structural rearrangements on the rheology of rennet-induced casein particle gels', *Adv Colloid Interface Sci*, 98, 25–50.

MODLER H W and KALAB M (1983), 'Microstructure of yogurt stabilised with milk proteins, *J Dairy Sci*, 66, 430–437.

MOTTAR J, BASSIER M, JONIAU M and BAERT J (1989), 'Effect of heat-induced association of whey proteins and casein micelles on yogurt texture', *J Dairy Sci*, 72, 2247–2256.

OZER B H, ROBINSON R K, GRANDISON A and BELL A E (1998), 'Gelation properties of milks concentrated by different techniques, *Int Dairy J*, 8, 793–799.

OZER B H, ROBINSON R K and GRANDISON A S (2002), 'Effect of elevation of total solids by ultra-filtration and reverse osmosis on thiol groups in milk', *Milchwissenschaft*, 57, 609–611.

PUVANENTHIRAN A, WILLIAMS R P W and AUGUSTIN M A (2002), 'Structure and visco-elastic properties of set yoghurt with altered casein to whey protein ratios', *Int Dairy J*, 12, 383–391.

ROBINSON R K (1981), 'Yoghurt – some considerations of quality', *Dairy Ind Int*, 46(3), 31–35.

ROBINSON R K (2000a), 'Formulations for yoghurt and other fermented milks', *Food Ingred Anal*, 22(2), 15–19.

ROBINSON R K (2000b), 'Yoghurt', in Robinson R K, Batt C A and Patel P D, *Encyclopedia of Food Microbiology*, London, Academic Press, 784–791.

ROBINSON R K and WILBEY R A (1998), *Cheesemaking Practice*, Gaithersburg, MD, Aspen Publishers.

ROBINSON R K, TAMIME A Y and WSZOLEK M (2002), 'Microbiology of fermented milks', in Robinson R K, *Handbook of Dairy Microbiology*, New York, John Wiley & Sons, 367–430.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

ROBINSON R K, LUCEY J A and TAMIME A Y (2006), 'Manufacture of yoghurt', in Tamime A Y, *Fermented Milks*, Oxford, Blackwell Publishing, 53–75.

ROGERS S A and MITCHELL G E (1994), 'The relationship between somatic cell count, composition and manufacturing properties of bulk milk', *Aust J Dairy Technol*, 49, 70–74.

SCOTT T W, FERGUSON K A, MCDONALD I W, BUCHANAN R A and HILLS G L (1970), 'Production of polyunsaturated milk fat in domestic ruminants', *Aust J Sci*, 32, 291–293.

SELLARS, R L (1991), 'Acidophilus products', in Robinson R K, *Therapeutic Properties of Fermented Milks*, London, Elsevier Applied Science, 81–116.

STELIOS K and EMMANUEL A (2004), 'Characteristics of set type yoghurt made from caprine or ovine milk and mixtures of the two', *Int J Food Sci Technol*, 39, 319–324.

TAMIME A Y (2002), 'Microbiology of starter cultures', in Robinson R K, *Handbook of Dairy Microbiology*, New York, John Wiley & Sons, 261–366.

TAMIME A Y and DEETH H C (1980), 'Yoghurt: technology and biochemistry', *J Food Prot*, 43, 939–977.

TAMIME A Y and MARSHALL V M E (1997), 'Microbiology and technology of fermented milks', in Law B A, *Microbiology and Biochemistry of Cheese and Fermented Milk*, London, Blackie Academic & Professional, 57–152.

TAMIME A Y and ROBINSON R K (2007), *Yoghurt – Science and Technology*, Cambridge, Woodhead Publishing.

TAMIME A Y, KALAB M and DAVIES G (1984), 'Microstructure of set-style yoghurt manufactured from cow's milk fortified by various methods', *Food Microstruct*, 3, 83–92.

TAMIME A Y, BRUCE J and MUIR D D (1993), 'Ovine milk: seasonal changes in microbiological quality of raw milk and yogurt', *Milchwissenschaft*, 48, 560–563.

VAN VLIET T and DENTENER-KIKKERT A (1982), 'Influence of the composition of the milk fat globule membrane on the rheological properties of acid milk gels', *Neth Milk Dairy J*, 36, 261–265.

VASBINDER A J, ALTING A C, VISSCHERS R W and DE KRUIF G G (2003), 'Texture of acid milk gels: formation of disulfide cross-links during acidification', *Int Dairy J*, 13, 29–38.

VLAHOPOULOU I, BELL A E and WILBEY A (1994), 'Starter culture effects on caprine yogurt fermentation', *J Soc Dairy Technol*, 47, 121–123.

WALSTRA P (1998), 'Relation between structure and texture of cultured milk products', in *Texture of Fermented Milk Products and Dairy Desserts*, Special Issue 9802, Brussels, International Dairy Federation, 9–15.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

# 16

# Milk quality requirements for cheesemaking

**S. Skeie, Norwegian University of Life Sciences, Norway**

**Abstract**: When using milk for cheesemaking, there are some requirements from the milk that need to be fulfilled; these factors, related to milk quality and milk treatment, are discussed in this chapter. A short introduction is given on the ranges of milk used for cheesemaking. It describes the effects of the microbial and chemical composition, and the effect of lactation, animal diet and seasonal variation on the cheesemaking properties of the cheese milk, focusing on cheese yield and cheese quality. The effects of different pre-treatments of milk before cheesemaking such as cold storage, heat treatments and membrane filtration are discussed.

**Key words:** milk quality, microbiology, cheese yield, cheese quality.

IP Address: 129.132.208.100

## 16.1 Introduction

By coagulating milk by the action of rennet or other coagulating agents and by partially draining off the whey, cheese is obtained (Codex Alimentarius, 2003). If the cheese curd is not ripened, fresh cheeses such as Cottage cheese or Quark are obtained, while if the cheese curd is pressed and salted, ripened soft or semi-hard and extra hard cheese varieties are obtained. Novel techniques such as ultrafiltration may be used for cheesemaking, but the cheese obtained should have similar physical, chemical and organoleptic characteristics as cheese made by traditional means. The whey protein/casein ratio of cheese should not exceed that of milk.

Milk of good quality for cheesemaking should have a low content of micro-organisms (preferably $<10^4$ cfu/ml milk) and somatic cells (<100,000/ml). To

© Woodhead Publishing Limited, 2010

obtain this, the milk should be produced by healthy animals and be as fresh as possible with as short a storage time as possible in the bulk tank. Cold storage may cause growth of psychrotrophic micro-organisms and leakage of calcium phosphate from the micelles. If storage of the milk is required, the milk should be thermized before storage to avoid growth of micro-organisms. The milk should have a high protein content, which has good cheesemaking properties. Preferably the milk protein should have the BB variants of $\beta$-lactoglobulin and $\kappa$-casein. The flavour of the milk should be of good quality, with no off-flavours. Pathogens and clostridia should be absent from the milk, but beneficial lactic acid bacteria may be present. The milk should contain no antimicrobial agents.

## 16.2 Range of milks used in cheesemaking

Different milk varieties are used for cheesemaking around the world; however, milk from cows is predominant. About 25% of the milks produced are used for cheese production, cows' milk representing 84% of the total world milk production (IDF, 2008). Other milks used for cheese production include buffalo, goat and ewe milk; these milks comprise 12, 2 and 1.2% of world milk production, respectively. The normal composition of these milks regarding protein, casein and ash is shown in Table 16.1. Ewe and buffalo milk have higher contents of protein and fat than the others and give, therefore, a much higher cheese yield (about 1.85- and 1.45-fold higher than cows' milk, respectively) than the other milk varieties (Alichanidis and Polychroniadou, 2008). The higher content of short chain fatty acids in milk from ewes and goats certainly influences the flavour of the cheese made from these milks.

In cow and ewe milk the protein constitutes 95% of the nitrogen-containing compounds in milk, while the remaining 5% is non-protein nitrogen (NPN). In goats' milk, the NPN represents around 6% of the total nitrogen (TN) (Kalantzopoulos, 1993), while in buffalo milk the NPN constitutes 2.3% of TN (Ahmad *et al.*, 2008). For traditional cheesemaking, the casein which constitutes 80% of the milk protein is utilized, and the remaining whey proteins will be lost. However, for cheese made by ultrafiltration also the remaining whey proteins,

IP Address: 129.132.208.100

**Table 16.1** Gross composition and pH of milk from cow, buffalo, goat and ewe

| Milk variety[a] | Protein | Casein | Ash | Fat | pH |
|---|---|---|---|---|---|
| Cow[2,3,4] | 3.2–3.8 | 2.4–2.7 | 0.7 | 4.1–4.3 | 6.7 |
| Buffalo[1,2] | 4.8–4.9 | 3.5 | 0.8 | 7.0–7.6 | 6.65–6.81 |
| Goat[2,4,6] | 2.6–3.7 | 2.11–2.93 | 0.8 | 3.0–5.2 | 6.6–6.7 |
| Ewe[5,6,7] | 4.9–6.1 | 4.73 | 0.9–1.0 | 6.3–8.2 | 6.4–6.7 |

[a] Origins and sources: [1]Chinese buffalo (Han *et al.*, 2007); [2]France (Ahmad *et al.*, 2008; Remeuf and Lenoir, 1986); [3]Sweden (Lindmark-Mansson *et al.*, 2003), [4]Norway (TINE, 2007; Devold *et al.*, 2000; Eknæs and Skeie, 2006); [5]Spain (Barron *et al.*, 2001); [6]Greece (Alichanidis and Polychroniadou, 2008); [7]General (Park *et al.*, 2007).

© Woodhead Publishing Limited, 2010

which represent 20% of the proteins, are included in the cheese yield. Casein is composed of the molecules $\alpha_{s1}$-, $\alpha_{s2}$-, $\beta$- and $\kappa$-casein; these differ in amino acid composition, phosphorylation and glycosylation. The caseins are polymorphic with several genetic variants, which also differ among the species. The number of genetic variants of the different caseins and whey proteins is shown in Table 16.2. The number of genetic variants seems also to vary among different breeds, and some species and breeds have been thoroughly investigated while others have not. We must therefore expect that Table 16.2 will be expanded as more research is conducted. The $\alpha_{s1}$-casein of goats' milk has a very broad variety with 16 known alleles, divided into strong alleles giving milk with a high content of $\alpha_{s1}$-casein and good cheesemaking properties, intermediate alleles, weak alleles and null alleles, where the milk completely lacks $\alpha_{s1}$-casein (Moioli *et al.*, 2007).

The four caseins are organized in casein micelles, where the hydrophobic caseins ($\alpha_{s1}$, $\alpha_{s2}$ and $\beta$) are situated in the core, while the more hydrophilic $\kappa$-casein is situated on the surface. $\kappa$-Casein contains carbohydrate groups, which make parts of this casein hydrophilic, resulting in a net negative charge. The C terminal hydrophobic part of $\kappa$-casein stands out from the surface of the micelle, stabilizing it from clotting with other casein micelles and preventing precipitation from the milk serum. Nanoclusters of calcium phosphate bind the casein molecules through esterification to serine (Ser) residues at the casein molecules. The $\alpha$-caseins have more Ser residues (8–12) than $\beta$-casein (5) and they are therefore more strongly bound to the casein micelle. The salts in milk are in a dynamic equilibrium (Fig. 16.1), with the colloidal calcium phosphate (CCP) situated within the micelles and $Ca^{2+}$ and $H(PO_4)^{2-}$ in the milk serum. In the milk serum, there is an equilibrium among the phosphates $H(PO_4)^{2-}$ and $H_2(PO_4)^{-}$ and $H^{+}$ (Walstra *et al.*, 2006).

Small ruminants produce milk with a much higher quantity of short chain fatty acids than large ruminants, which have a higher percentage of long chain fatty acids. The composition of fatty acids in cheese milks is highly dependent on the feeding of the animal. A quite large variation within the species might therefore be found. The normal composition of fatty acids in milk from these animals is shown in Table 16.3.

IP Address: 129.132.208.100

## 16.3 Effects of milk on cheesemaking, yield and quality

### 16.3.1 Microbial quality of milk

Milk from healthy cows is practically sterile when it is drawn from the udder; however, as soon as it leaves the udder it is contaminated by various micro-organisms. Milk used for cheesemaking must be of good microbiological quality, with a low total count of bacteria, absence of pathogenic and detrimental bacteria, and a low count of psychrotrophic bacteria, which produce heat-resistant proteases and lipases that may reduce yield and cause undesirable flavours in the ripened cheese.

© Woodhead Publishing Limited, 2010

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

**Table 16.2** Known genetic variants of caseins and whey proteins in milk from cow, buffalo, goat and ewe

| Milk variety[a] | $\kappa$-Casein | $\alpha_{s1}$-Casein | $\alpha_{s2}$-Casein | $\beta$-Casein | $\beta$-Lactoglobuline | $\alpha$-Lactalbumin |
|---|---|---|---|---|---|---|
| Cow[3] | 11<br>A–C, E, F$^1$, F$^2$, G$^1$, G$^2$, H–J | 8<br>A–H | 4<br>A–D | 12<br>A$^1$, A$^2$, A$^3$, B–G, H$^1$, H$^2$, I | 11<br>A–J, W | 3<br>A–C |
| Buffalo[4,5,6,7] | 1 | 2 | 1 | 2<br>A, B | 1 | 2<br>A, B |
| Goat[1] | 14<br>A, B$^1$, B$^2$, C, C$^1$, D–L, M | 16<br>Strong: A, B$^1$, B$^2$, B$^3$, B$^4$, C, H, L, M<br>Intermediate: E, I<br>Weak: F, G<br>Null: 0$^1$, 0$^2$, N | 7<br>A–F, 0 | 6<br>A–C, 0$^1$, 0$^2$, 0$^3$ | 2<br>A, B | |
| Ewe[1,2] | 2 | 5<br>A–E | 2<br>A, B | | 3<br>A–C | 3<br>A–C |

[a] Sources: [1]Moioli *et al.*, 2007; [2]Amigo *et al.*, 2000; [3]Farrell *et al.*, 2004; [4]Chianese *et al.*, 2004; [5]Klotz *et al.*, 2000; [6]Ferranti *et al.*, 1999; [7]D'Ambrosio *et al.*, 2008.

**Table 16.3** Fatty acid composition (min–max %) of different cheese milks

| Animal species[a] | Short chain fatty acids, C4–C12 | Medium chain fatty acids, C13–C16 | Long chain fatty acids, C17–C22 |
|---|---|---|---|
| Cow[1] | 8–22 | 32–54 | 31–50.5 |
| Buffalo[2] | 2.6 | 42.7 | 54.8 |
| Goat[3] | 19.3–26.1 | 33.1–50.8 | 25.5–50.0 |
| Ewe[3] | 17.1–25.6 | 35.0–43.3 | 32.1–42.0 |

[a] Sources: [1]Jensen, 2002; [2]Shahin *et al.*, 1987; [3]Park *et al.*, 2007.

*Lactic acid bacteria*

In a dairy environment mesophilic lactic acid bacteria will always be present, and in fresh raw milk with low bacterial counts, a mesophilic microflora is dominating. However, as the milk is cooled during bulk storage, the growth of these bacteria is restricted. During cheesemaking, lactic acid bacteria are normally added as starters, but during cheese ripening, the presence of non-starter lactic acid bacteria (NSLAB) in the milk appears to be of importance for the final cheese quality. Some NSLAB survive pasteurization and may influence the ripening process. Mesophilic lactobacilli are the main species of NSLAB in cheese; they seem to be of importance for amino acid metabolism, which contributes to flavour compounds during cheese ripening, but NSLAB have also been connected to undesirable sensory properties in ripened cheeses (Beresford and Williams, 2004; Fox *et al.*, 2004). Østlie *et al.* (2004, 2005) showed that the flora of NSLAB in Norvegia and Präst cheeses varied with cheese age and between different dairies in Norway and Sweden. The variation between the dairy plants must, therefore, be attributed either to the specific dairy plant or to the milk from their supply regions.

IP Address: 129.132.208.100

*Pathogenic bacteria*

Pathogenic bacteria should be absent from the cheese milk; this is of utmost importance when cheese is produced from unpasteurized milk. *Salmonella enterica*, *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus* are associated with raw milk (Donnelly, 2004). These pathogens are killed during pasteurization, and Bachmann and Spahr (1995) found that *Salmonella enterica*, *Escherichia coli* and *Staph. aureus* died out during ripening in hard and semi-hard cheese varieties made from raw milk due to low water activity and low pH. However, Schlesser *et al.* (2006) observed that Cheddar cheese made from raw milk inoculated with a cocktail of *Escherichia coli* O157:H7 strains showed a decrease of only 1 and 2 log after ripening at 7ºC for 60 and 120 days, respectively. When *Escherichia coli* O157:H7 was inoculated at $10^3$ or more cfu/ml milk, viable cells were still found after 360 days of ripening in some cheeses. *Salmonella*, *Listeria* and *Escherichia coli* have developed systems to combat stress, and may develop an acid tolerance response under acidic conditions (Gahan and Hill, 1999; Leyer *et al.*, 1995; Foster, 2001). Acid-adapted

© Woodhead Publishing Limited, 2010

strains of *Salmonella* Typhimurium were found to survive in Mozzarella and Cheddar cheese under conditions which were supposed to be very unfavourable (Leyer and Johnson, 1992). When soft cheeses are produced from raw milk, the absence of pathogens is a premise. With the knowledge we now have about stress adaption of pathogenic bacteria, this should also be assumed for semi-hard cheeses.

*Staphylococcus aureus* produces heat-stable enterotoxins, which, when ingested, cause symptoms within a short period of time (2 to 6 h). These symptoms include nausea, vomiting and diarrhoea (Jørgensen *et al.*, 2005). In milk used for raw milk cheeses, the presence of *Staph. aureus* should be of concern, as this bacterium usually is present in milk from animals with subclinical mastitis, and it has been shown to survive in semi-hard cheese during ripening (Bachmann and Spahr, 1995; Spahr and Url, 1994). Jørgensen *et al.* (2005) found that 10 out of 11 cows on a Norwegian farm were carriers of *Staph. aureus*; however, *Staph. aureus* was also found on the farmer, on equipment, in the environment and on the raw milk cheese produced on the farm.

*Mycobacterium paratuberculosis* (MAP) causes paratuberculosis or Johne's disease in cattle and has also been connected to Crohn's disease in humans. Herthnek *et al.* (2008) found that milk from 68% of 56 investigated Danish herds were positive for MAP. Nielsen and Toft (2009) calculated the prevalence among cattle in Europe to be approximately 20%. The research evidence for the ability of MAP to survive pasteurization has been contradictory; however, it is now generally recognized that MAP does not survive proper pasteurization (Griffiths, 2009). If MAP is present in the cheese milk, it may survive in cheese during ripening (Clark *et al.*, 2006; Donaghy *et al.*, 2003; Stephan *et al.*, 2007; Spahr and Schafroth, 2001).

IP Address: 129.132.208.100

*Detrimental bacteria*

Sporeforming bacteria, such as *Clostridium tyrobutyricum*, or psychrotrophic bacteria which produce heat-stable proteolytic and lipolytic enzymes, may be detrimental for cheese quality and the load of these bacteria in cheese milk should be as low as possible.

The anaerobe *Clostridium tyrobutyricum* produces butyric acid, $CO_2$ and $H_2$ from lactic acid. In cheeses with eyes, which normally have a pH > 5.3 within the first 24 h, it may cause late blowing of the cheese in the hot ripening room, as it grows well under these conditions. But also in cheeses with a closed texture, which often have a 24 h pH < 5.2, these bacteria may produce flavours rendering the cheese inedible. Of utmost importance to avoid the presence of these bacteria are 'clean silage' without spores, sanitary conditions in the cowshed and good hygienic conditions during milking (Walstra *et al.*, 2006).

The total level of bacteria in raw milk is normally in the range of $10^4$–$10^6$ cfu/ml (Sørhaug and Stepaniak, 1991). When the microflora of the milk exceeds $10^4$ cfu/ml, the flora is dominated by Gram-negative and lactose-negative psychrotrophic bacteria. In newly drawn milk, 1–10% of the total bacterial count consists of psychrotrophic bacteria, while these dominate totally after 2 to 3 days

© Woodhead Publishing Limited, 2010

of storage. Ternstrøm *et al.* (1993) showed that the psychrotrophic flora in Norwegian and Swedish raw milk totalled 80% of the microflora when the cfu/ml exceeded $10^7$, with *Pseudomonas* as the dominating genus. The average generation time of psychrotrophic bacteria at 4ºC is between 4.5 and 11 hours (Sørhaug and Stepaniak, 1991) and, according to Suhren (1989), the critical storage time for raw milk is between 60 and 72 hours when the temperature is between 2 and 4ºC. Thermization is often applied upon arrival of the milk at the dairy plant, when storage of the raw milk is needed for more than 2 days. As most of the psychrotrophic bacteria are killed by pasteurization, these Gram-negative bacteria do not represent a quality problem as such in milk, but they produce heat-resistant extracellular proteases and lipases which may cause severe problems (Mottar, 1989) as the conditions in milk are conducive for proteolytic activity. Kohlmann *et al.* (1991) found that proteases from *P. fluorescens* M3/6 had activity on $\alpha$-, $\beta$- and $\kappa$-caseins. Frohbieter *et al.* (2005) showed that proteases produced by *P. fluorescens* M3/6 stimulated plasminogen activators, resulting in the transformation of plasminogen to plasmin.

### 16.3.2 Somatic cell count

Milk with a high somatic cell count (SSC) (>500,000 cells/ml milk) reduces cheese yield (Auldist *et al.*, 1996), and such milk is associated with higher proteolytic activity, lower concentration of fat and casein and a higher content of whey proteins, especially serum albumin and immunoglobulin. The somatic cells contain a plasmin activator that converts plasminogen to plasmin in the mammary gland (Lucey and Kelly, 1994). According to de Rham and Andrews (1982), plasmin totalled one-third of the total protease activity in milk with high SSC. Plasmin degrades mainly $\beta$- and $\alpha_{s2}$-casein into peptides, and plasmin might be slightly active at 5ºC (van den Berg *et al.*, 1996). Based on results showing a marked decrease in cheese yield at SSC > 100,000/ml milk, Barbano *et al.* (1991) suggested the upper limit for SSC for cheese milk to be 100,000/ml. In addition to decreased cheese yield, the increased proteolytic activity in milk caused by increased levels of SSC has been shown to influence the cheese composition by increasing the moisture content, decreasing the protein content, and increasing proteolysis (Cooney *et al.*, 2000; Grandison and Ford, 1986). Cheese made from milk with high numbers of SSC exhibited a decreased firmness and elasticity and an increased stickiness and off-flavour (Grandison and Ford, 1986). Auldist *et al.* (1996) found the same effects of high SSC in the cheese milk, though the effect of high SSC was more detrimental for cheese quality in late lactation milk than in early lactation milk.

### 16.3.3 Lactation

The concentrations of the various milk constituents vary during lactation (Lucey and Kelly, 1994), and the content of fat and protein is much higher in colostrum milk than in normal milk. From around lactation week 5, it has been shown that

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

the fat and protein content of the milk is at its minimum with a steady increase during further lactation, while the concentration of lactose decreases slowly during lactation (Fox *et al.*, 2000). Guinee *et al.* (2007) showed that the protein content of milk increased during lactation with a similar increase of cheese yield. At very late lactation the protein content of the milk dropped and thereby also the cheese yield; however, it was not decreased with reference to the levels of fat and protein. As very late lactation milk normally contains more serum proteins, it exhibits more hydrophilic properties and a higher milk pH; in addition, when the content of SSC is high and the content of casein low, the coagulation property of the milk is reduced. This results in a weak curd with poor syneresis of the casein network and a cheese with higher moisture content (Auldist *et al.*, 1996). It has been shown that if the diet is balanced with the lactation, high quality milk may be produced during the entire lactation (Guinee *et al.*, 2007; Auldist *et al.*, 1996; Kefford *et al.*, 1995). Late lactation milk has previously been considered inferior for cheesemaking, since the numbers of SSC increase and the content of whey protein increases at the expense of casein; however, Kefford *et al.* (1995) showed that if the cows were offered a high quality diet these changes did not occur in late lactation milk.

Plasmin is derived from plasminogen by the action of a plasminogen activator in milk and the levels of plasmin, plasminogen and plasminogen activator increase in milk during lactation (Baldi *et al.*, 1996). The content of plasminogen peaks during the fifth month of lactation, while the ratio of plasminogen to plasmin decreases during lactation. These results may indicate an increased conversion of plasminogen to plasmin during lactation.

IP Address: 129.132.208.100

### 16.3.4 Animal diet

The animal diet will certainly influence the cheesemaking properties of the milk. A diet containing a high content of concentrate will increase the protein content of the milk (Coulon and Rémond, 1991), and by that directly influence the cheese yield. The fat content and fat composition of the milk depend on the physiological status of the animal and the types of fatty acids given in the feed (Urbach, 1990; Eknæs and Skeie, 2006). Feeding strategies that alter the milk fat composition in favour of long chain unsaturated fatty acids have been the recent focus of milk production research. When cheese has been made from these milks, however, contradictory results have been found. While Allred *et al.* (2006) and Lightfield *et al.* (1993) found no influence on cheese quality when cheese was made from milk with altered milk fat composition, Jaros *et al.* (2001) obtained cheese with reduced firmness when using such milk, which is an interesting aspect when dealing with low fat cheese. When making cheese from milk produced by cows fed red clover (Steinshamn and Thuen, 2008), a higher proportion of long chain unsaturated fatty acids was obtained in the milk fat and the texture of a fat-reduced cheese was improved (Svanborg, 2006).

Several experiments have also shown the influence of the botanical composition of the pasture on the composition of cheese, with changed flavour and

© Woodhead Publishing Limited, 2010

texture of the cheese made from milk produced at higher altitudes (Martin *et al.*, 2005; Bugaud *et al.*, 2001, 2002; Noni and Battelli, 2008). The sensory properties of Comté cheese have been shown to differ with soil variations (georegions) and by the botanical diversity of the pastures (Monnet *et al.*, 2000). Cheese made on the Swedish island of Øland during the summer had a composition of organic acids, amino acids and volatile components that differed from that of cheese made on the Swedish mainland (Skeie, unpublished results). During summer the cows on Øland graze a pasture that is high in allium plants. Such plants are normally considered detrimental for grazing dairy cows, as an onion flavour is very rapidly released during rumen fermentation and is transferred to the milk via the lungs and the blood of the cow (Urbach, 1990).

It has been known by cheesemakers that cheese made from milk produced by animals fed silage or fresh pasture is yellower and often softer than cheese made from animals fed hay. However, few differences have been found between cheeses when comparing the effect of feeding within the same season, while larger differences have been found between seasons (Verdier-Metz *et al.*, 1998; Agabriel *et al.*, 2004). Verdier-Metz *et al.* (1998) showed that Saint-Nectaire cheeses made from milk of cows fed silage were yellower and more bitter than cheese made from milk of cows fed hay produced from the same sward, while other chemical and sensory properties did not differ between the treatments. However, when comparing Saint-Nectaire cheese made from winter milk and pasture milk, significant differences were found in the texture and flavour properties of the cheese (Coulon *et al.*, 2004).

IP Address: 129.132.208.100

### 16.3.5 Genetic variants

Milk from Jersey cows has normally a higher protein and fat content than milk from Friesian cows and has, therefore, better cheesemaking properties. According to Auldist *et al.* (2004) this was related to a higher content of total solids in the milk, and was not connected to the genotype of $\kappa$-casein. Several studies have shown that milk with the BB variant of $\beta$-lactoglobulin (Lg) and $\kappa$-casein gives the best curd firmness and cheese yield (van den Berg *et al.*, 1996; Walsh *et al.*, 1998; Hallén *et al.*, 2007). A higher casein to total protein ratio, and a higher casein content, are obtained in milk with the BB variant of $\beta$-Lg. Milk with the BB variant of $\kappa$-casein is associated with a higher casein level and higher $\kappa$-casein content, resulting in smaller micelles, a shorter rennet coagulation time and a higher curd firmness. However, this is not always the case, as Mayer *et al.* (1997) showed that the composite milk protein phenotype effects were also of importance for the cheese yield. Milk with the combination $\beta$-casein $A^2A^2$, $\kappa$-casein AA and $\beta$-Lg AA gave 30% lower cheese yield compared to milk with the BB variants of $\kappa$-casein and $\beta$-Lg. However, a higher cheese yield was obtained from milk with $\beta$-casein $A^2B$, $\kappa$-casein AA and $\beta$-Lg AA than from milk with the combination $\kappa$-casein BB, $\beta$-Lg BB and $\beta$-casein $A^2B$. Another conclusion from this work was that the perfect combination does not

© Woodhead Publishing Limited, 2010

exist, because no genotypic combination gave, together, the lowest fat in whey, lowest cheese fines, highest cheese yield and highest proteolysis.

Wedholm *et al.* (2006) found that milk samples from individual Swedish and Danish cows causing a weak coagulum had a low concentration of $\kappa$-casein and a low amount of $\kappa$-casein in proportion to total casein. Milk having the AA genotype of $\kappa$-casein had lower concentrations of $\kappa$-casein than milk having the AB genotype. They also found a high frequency of $\kappa$-casein AE in milk with poor coagulation properties. Based on these results, Wedholm *et al.* (2006) concluded that milk for cheesemaking should be selected for high concentrations of $\alpha_{s1}$-, $\beta$- and $\kappa$-casein and $\beta$-Lg B. The frequency of the E variant of $\kappa$-casein increased in Sweden and Finland during the 1970s and 1980s, as milk containing this variant has been associated with increased milk yield.

Coulon *et al.* (2004) reviewed the effect of genetic variants of the caseins on the sensory properties of various cheeses; they report differences in the sensory attributes of cheeses made from cows' milk differing in the genetic variants of $\beta$-casein and from goats' milk differing in the genetic variants of $\alpha_{s1}$-casein.

### 16.3.6 Seasonal variation

In regions with changing climate or where milk production has a seasonal pattern, large seasonal variations may be found in milk composition. When milk production has a seasonal pattern, these changes can also be partly attributed to lactation. In Australia, New Zealand and Ireland, there is a very clear seasonal pattern due to lactation (Kefford *et al.*, 1995). However, the quality of feed may be more important than the stage of lactation for the cheesemaking properties of the milk (Kefford *et al.*, 1995). Van den Berg *et al.* (1996) state that calcium, magnesium, inorganic phosphate and citrate show a similar seasonal trend in Dutch milk, and that the ratio of colloidal minerals and citrate was highest in March/April and lowest in August. In Scandinavia, the lactation curve is mostly straightened out throughout the year, but in the north of Scandinavia there is a very clear seasonal pattern in the milk composition due to climate. Lindmark-Mansson *et al.* (2003) found seasonal variation in most of the 94 parameters analysed, which was correlated to outdoor grazing from May till October. In Norway, Johansen *et al.* (2002) found that the amount of protein in whey was at its highest during summer and winter time with a drop during spring and autumn, while the content of urea (non-protein nitrogen) was lower during summertime.

IP Address: 129.132.208.100

## 16.4 Influence of milk preparation for its cheesemaking properties and for cheese quality

### 16.4.1 Influence of cold storage

Cold storage is not considered to be beneficial for the cheesemaking properties of milk, and therefore many of the traditional European cheeses with protection

© Woodhead Publishing Limited, 2010

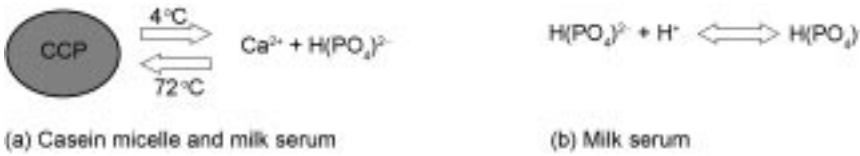



**Fig. 16.1** Balance of colloidal calcium phosphate (CCP) and the milk serum phosphates (a) between the casein micelle and the milk serum, and (b) in the milk serum.

of origin (PDO or AOP) are made from newly drawn milk. Basically, it is the equilibrium of the milk salts that is disturbed during cold storage and this may create a problem for further processing into cheese. In the casein micelle, calcium phosphate is supersaturated and is in equilibrium with dissociated ions in the milk serum (Fig. 16.1). At low temperatures, as used during cold storage of milk, the solubility of $Ca^{2+}$ in the milk serum increases. Colloidal calcium phosphate (CCP) is therefore drawn from the casein micelles to the serum to recover equilibrium. At first the loosely bound CCP is removed, which does not affect the micelle structure significantly, but when the more strongly bound CCP starts to leave the micelle, the micellar structure opens up, resulting in weakening of the CCP bonds between the casein molecules. $\beta$-Casein has few serine-phosphate groups and is mainly bound to the micelle by hydrophobic bonds, which are weakened at low temperature, and it therefore starts to leak from the micelle at low temperatures (Walstra *et al.*, 2006).

Reimerdes and Klostermeyer (1976) observed that the concentration of $\beta$-casein increased from 0.25 to 0.9% in the milk serum after 2 h storage at 5ºC. However, they also observed that most of the leakage occurred at the start of the cooling period, as the concentration of $\beta$-casein in the milk serum increased to only 1% after 20 h at 5ºC. As the leakage of CCP and $\beta$-casein are reversed at elevated temperatures, for example those achieved during pasteurization, only minor effects of this leakage can be observed on the renneting properties of milk during cheese production from pasteurized milk. However, the $\beta$-casein may be exposed to proteolytic action from indigenous milk enzymes or proteases produced by psychrotrophic micro-organisms during cold storage, and the proteolytic products will not be recovered by the casein micelles on heating but will be lost with the whey. Proteolytic action on $\beta$-casein during cold storage of milk will therefore result in a reduced cheese yield. It is therefore extremely important that the somatic cell count and the microbial contamination are kept as low as possible in milk used for cheese production, especially when it is cold stored. In order to avoid the proteolytic degradation of $\beta$-casein during cold storage of milk, the dairy industry often thermizes the milk as they receive it, if the milk is going to be stored before cheesemaking. However, a better strategy, if possible, would be to store the milk for a minimal amount of time before cheesemaking.

IP Address: 129.132.208.100

### 16.4.2 Influence of heat treatment

As the mineral balance, the whey proteins and the micro-organisms of the milk are influenced by heat treatment, this will also influence the cheesemaking

© Woodhead Publishing Limited, 2010

properties of the milk. A rearrangement of the casein micelle occurs as CCP and $\beta$-casein return to the casein micelle, causing a rearrangement of the casein micelle. The cheesemaking characteristic of cooled milk is therefore improved by heat treatment. However, as milk is heat treated, the globular proteins of the milk, i.e., the whey proteins, will start to denature, and this may adversely affect the cheesemaking properties of the milk. $\beta$-Lactoglobulin constitutes around 60% of the whey proteins and associates with $\kappa$-casein as it denatures. The rennet will have difficulties entering the cleaving site of $\kappa$-casein (phe-met; 105–106) when it is covered with denatured $\beta$-lactoglobulin, and the renneting properties of the milk will deteriorate. The denaturation temperature of $\beta$-lactoglobulin is 78ºC; normal milk pasteurization at 72ºC for 15 s will, therefore, normally have only a minor influence on the renneting properties of cheese milk (Walstra *et al.*, 2006).

It has been shown that the microflora of cheese is strongly influenced by the milk treatment (Dasen *et al.*, 2003; Grappin and Beuvier, 1997), and that the composition of the products of cheese ripening, such as the content of free amino acids, volatiles and the sensory properties of cheese, are significantly influenced by the extent of the heat treatment applied to the milk (Skeie and Ardo, 2000; Buchin *et al.*, 1998; Bachmann *et al.*, 1998).

### 16.4.3 Influence of membrane filtration

Membrane filtration has, during the last 40 years, become a common pre-treatment of milk before cheesemaking. Microfiltration (MF) has primarily been used for removal of bacteria and spores from skimmed milk before cheese-making by the use of ceramic membranes with a pore size of 1–10 $\mu$m, separating molecules with a molecular weight higher than 200,000 Da. The spores and bacteria will be retained in the MF retentate. If the retentate is subjected to UHT treatment and added to the cheese milk (Bactocatch process), the cheese milk will contain whey proteins and the water-binding properties of the cheese will be increased. However, if the retentate is not included in the cheese milk, MF has a minor influence on the cheesemaking properties of the milk. The cheese will, however, contain only micro-organisms added after milk treatment and the direction of the ripening is thus affected compared to non-MF milk.

Ultrafiltration (UF) has been used for concentration of the milk proteins, using membranes with a pore size of $10^{-4}$–$10^{-3}$ $\mu$m, separating molecules with a molecular weight between 1000 and 200,000 Da. Ultrafiltration is used for either standardization or full concentration of cheese milk, obtaining a liquid pre-cheese. By using UF for standardization, the protein content is increased to <5%, and at this degree of concentration the influence on cheese ripening and quality is minimal. The cheese production will, however, be more standardized and the cheesemaking properties of the milk will be improved. Cheese made from full concentrated milk also contains whey proteins and an increased cheese yield is obtained. The ripening of cheese made from full concentrated milk is

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

altered in many ways; the most apparent is the reduced proteolysis. The mechanism is not clearly understood, but it is connected to increased mineral content and the presence of whey proteins. If milk is ultrafiltered at its normal pH, the mineral salts bound to the casein micelles are concentrated in the same proportion as the protein, resulting in an increased buffering capacity of the UF retentate. This influences the rennet coagulation and acidification kinetics of the milk and cheese (Mistry and Maubois, 2004).

### 16.4.4 Influence of novel techniques

*High pressure treatment of cheese milk*

High pressure treatment of cheese milk is of interest since this treatment is capable of inactivating micro-organisms and bacteriophages. However, this treatment also causes changes to the cheese milk depending on the pressure used: at 100 MPa (10 bar) $\beta$-lactoglobulin is denatured; at pressures >250 MPa the casein micelles aggregate, and the rennet clotting time (RCT) of the milk is reduced; at pressures >400 MPa $\alpha$-lactalbumin is denatured and the casein micelles start to dissociate causing an increased RCT; at pressures between 600 and 800 MPa a 25% increase in cheese yield is obtained, caused by incorporation of denatured whey proteins and increased moisture retention. The fact that this treatment has to be applied in a batch or semi-continuous system limits the implementation of this technology in the cheesemaking industry (Kelly *et al.*, 2008).

IP Address: 129.132.208.100

*Treatment of cheese milk with carbon dioxide*

Carbon dioxide ($CO_2$) is commonly used by the food industry to prevent food spoilage and to increase shelf-life, as it has an inhibitory effect against many food spoilage micro-organisms. The solubility of $CO_2$ in milk is high and it causes a pH decrease in the milk (Hotchkiss *et al.*, 2006). Rajagopal *et al.* (2005) observed reduced growth of Gram-negative bacteria in raw milk when stored under $CO_2$ pressure. Eie (1994) found that carbonation of goat and cow milk inhibited psychrotrophic growth and proteolysis in milk during storage. The carbonated milk had reduced rennet clotting time and gel formation time, most probably due to the reduced pH of the milk. Jarlsberg cheese made from stored carbonated milk exhibited an improved texture and flavour compared to cheese made from stored control milk, which developed a strong rancid flavour. When adding $CO_2$ to the cheese milk after pasteurization, Nelson *et al.* (2004a, 2004b) observed a lower pH at whey drainage, a reduced time from rennet addition to whey drainage, a lower fat retention, a reduced yield and an increased proteolysis compared to cheese made from control milk.

*Novel use of microfiltration*

As ceramic membranes with reduced pore size (0.1–0.2 $\mu$m) and polymeric spiral-wound membranes have been developed, microfiltration may now be used to concentrate casein and to separate the whey proteins from the milk before

© Woodhead Publishing Limited, 2010

cheesemaking (Kelly *et al.*, 2008). By combining microfiltration, ultrafiltration and diafiltration, the content of casein, whey proteins, minerals and lactose in the cheese milk may be regulated before cheesemaking; in this way the washing step during cheesemaking may be omitted (Heino *et al.*, 2008). Traditionally, microfiltration is performed at around 50ºC, which may increase the risk of microbial growth and denaturation of whey proteins (Govindasamy-Lucey *et al.*, 2007), in addition to the costs of warming the milk. Cold microfiltration represents a possibility to avoid these risks.

## 16.5 Future trends

The future trends when it comes to milk quality requirements for cheesemaking will most probably be related to milk composition but also to novel techniques for milk pretreatment. As the importance of the genomics of the cow for milk composition has been elucidated, breeding programmes will focus on the functional properties of milk. We can therefore expect that milk production will be more specialized towards the products produced from the milk. Previously, feeding programmes have focused on milk yield and gross composition of the milk, but in the future they will focus on the influence of the feed on composition of minor components, to increase the content of, for instance, long chain fatty acids, conjugated linoleic acid, and compounds giving or being precursors of characteristic flavour notes of the ripened cheese. Most probably the cheese plants will increase their focus on minimizing the cold storage of milk before cheesemaking.

The cheese industry has started to use non-starter lactic acid bacteria often isolated from raw milk cheeses to produce the 'wild flavour' of cheeses made from pasteurized and microfiltered milk. This use will increase as new and more promising isolates are found. In this way cheeses with the characteristic flavour of a raw milk cheese may be produced safely with reduced risk of the growth of pathogenic bacteria. The dairy industry has previously focused on cheese yield and, as this is essential for the business of cheese production, this will still be an area of interest. In the future we will see greater use of novel pretreatment techniques of milk, which will contribute to increased cheese yield and provide a more stable cheese quality; in this aspect membrane filtration techniques are of special interest.

## 16.6 Sources of further information and advice

Several excellent reviews have been written on this topic, focusing on different factors of importance for milk quality of sheep's milk (Pulina *et al.*, 2006), of goat's milk (Chilliard *et al.*, 2003) and of goat's and ewe's milk (Kalantzopoulos, 1993); the relationship between ruminant management and the sensory characteristics of cheese (Coulon *et al.*, 2004); factors important for cheese yield

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

and the influence of the feed for milk quality (Lucey and Kelly, 1994); the effect of high temperatures on the casein micelles (Singh, 1988); the implications of milk pasteurization on the manufacture and sensory quality of ripened cheese (Grappin and Beuvier, 1997); pretreatment of cheese milks (Kelly *et al.*, 2008); the effect of adding $CO_2$ to dairy products (Hotchkiss *et al.*, 2006); and membrane filtration of cheese milk (Saboya and Maubois, 2000).

As a textbook for students in dairy science the book *Dairy Science and Technology* by Walstra *et al.* (2006) is highly recommended. As a textbook for cheese science students, the book *Fundamentals of Cheese Science* by Fox *et al.* (2000) is advised. The two volumes of the comprehensive books *Cheese: Chemistry, Physics and Microbiology* edited by Fox *et al.* (2004) are mandatory for the cheese scientist. The book *Cheese Problems Solved* by McSweeney (2007) will be a helpful tool for cheesemakers and scientists who are looking for answers to problems related to cheese quality.

## 16.7 References

AGABRIEL, C., MARTIN, B., SIBRA, C., BONNEFOY, J.-C., MONTEL, M.-C., DIDIENNE, R. and HULIN, S. (2004), Effect of dairy production systems on the sensory characteristics of Cantal cheeses: a plant-scale study. *Animal Research*, 53, 221–234. doi: 10.1051/animres:2004013

AHMAD, S., GAUCHER, I., ROUSSEAU, F., BEAUCHER, E., PIOT, M., GRONGNET, J. F. and GAUCHERON, F. (2008), Effects of acidification on physico-chemical characteristics of buffalo milk: A comparison with cow's milk. *Food Chemistry*, 106, 11–17. doi: 10.1016/j.foodchem.2007.04.021

ALICHANIDIS, E. and POLYCHRONIADOU, A. (2008), Characteristics of major traditional regional cheese varieties of East-Mediterranean countries: a review. *Dairy Science and Technology*, 88, 495–510. doi: 10.1051/dst:2008023

ALLRED, S. L., DHIMAN, T. R., BRENNAND, C. P., KHANAL, R. C., MCMAHON, D. J. and LUCHINI, N. D. (2006), Milk and cheese from cows fed calcium salts of palm and fish oil alone or in combination with soybean products. *Journal of Dairy Science*, 89, 234–248.

AMIGO, L., RECIO, I. and RAMOS, M. (2000), Genetic polymorphism of ovine milk proteins: its influence on technological properties of milk – a review. *International Dairy Journal*, 10, 135–149. doi: 10.1016/S0958-6946(00)00034-0

AULDIST, M. J., COATS, S., SUTHERLAND, B. J., MAYES, J. J. and MCDOWELL, G. H. (1996), Effects of somatic cell count and stage of lactation on raw milk composition and the yield and quality of Cheddar cheese. *Journal of Dairy Research*, 63, 269–280. doi: 10.1017/S0022029900031769

AULDIST, M. J., JOHNSTON, K. A., WHITE, N. J., FITZSIMONS, W. P. and BOLAND, M. J. (2004), A comparison of the composition, coagulation characteristics and cheesemaking capacity of milk from Friesian and Jersey dairy cows. *Journal of Dairy Research*, 71, 51–57. doi: 10.1017/S0022029903006575

BACHMANN, H. P. and SPAHR, U. (1995), The fate of potentially pathogenic bacteria in Swiss hard and semihard cheeses made from raw milk. *Journal of Dairy Science*, 78, 476–483.

BACHMANN, H. P., BANKS, J., BERESFORD, T., BÜTIKOFER, U., GRAPPIN, R., LAVANCHY, P.,

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

LINDBLAD, O., MCNULTY, D., MCSWEENEY, P. L. and SKEIE, S. (1998), Interlaboratory comparison of cheese making trials: model cheeses made from raw, pasteurized and microfiltered milks. *Lebensmittel-Wissenschaft und -Technologie*, 31, 585–593. doi:10.1006/fstl.1998.0419

BALDI, A., SAVOINI, G., CHELI, F., FANTUZ, F., SENATORE, E., BERTOCCHI, L. and POLITIS, I. (1996), Changes in plasmin–plasminogen–plasminogen activator system in milk from Italian Friesian herds. *International Dairy Journal*, 6, 1045–1053. doi:10.1016/0958-6946(95)00075-5

BARBANO, D. M., RASMUSSEN, R. R. and LYNCH, J. M. (1991), Influence of milk somatic cell count and milk age on cheese yield. *Journal of Dairy Science*, 74, 369–388.

BARRON, L. J. R., FERNÁNDEZ DE LABASTIDA, E., PEREA, S., CHÁVARRI, F., DE VEGA, C., SOLEDAD VICENTE, M., ISABEL TORRES, M., ISABEL NÁJERA, A., VIRTO, M., SANTISTEBAN, A., PÉREZ-ELORTONDO, F. J., ALBISU, M., SALMERÓN, J., MENDÍA, C., TORRE, P., CLEMENTE IBÁÑEZ, F. and DE RENOBALES, M. (2001), Seasonal changes in the composition of bulk raw ewe's milk used for Idiazabal cheese manufacture. *International Dairy Journal*, 11, 771–778. doi:10.1016/S0958-6946(01)00120-0

BERESFORD, T. and WILLIAMS, A. G. (2004), The microbiology of cheese ripening. In Fox, P. F., McSweeney, P. L., Cogan, T. M. and Guinee, T. P (eds), *Cheese: Chemistry, Physics and Microbiology*, 3rd edn. London, Elsevier.

BUCHIN, S., DELAGUE, V., DUBOZ, G., BERDAGUE, J. L., BEUVIER, E., POCHET, S. and GRAPPIN, R. (1998), Influence of pasteurization and fat composition of milk on the volatile compounds and flavor characteristics of a semi-hard cheese. *Journal of Dairy Science*, 81, 3097–3108.

BUGAUD, C., BUCHIN, S., HAUWUY, A. and COULON, J. B. (2001), Relationships between flavour and chemical composition of Abondance cheese derived from different types of pastures. *Lait*, 81, 757–773. doi: 10.1051/lait:2001162

BUGAUD, C., BUCHIN, S., HAUWUY, A. and COULON, J. B. (2002), Flavour and texture of cheeses according to grazing type: the Abundance cheese. *Productions Animales*, 15, 31–36.

CHIANESE, L., CAIRA, S., LILLA, S., PIZZOLONGO, F., FERRANTI, P., PUGLIANO, G. and ADDEO, F. (2004), Primary structure of water buffalo $\alpha$-lactalbumin variants A and B. *Journal of Dairy Research*, 71, 14–19. doi:10.1017/S0022029903006551

CHILLIARD, Y., FERLAY, A., ROUEL, J. and LAMBERET, G. (2003), A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. *Journal of Dairy Science*, 86, 1751–1770.

CLARK, D. L., ANDERSON, J. L., KOZICZKOWSKI, J. J. and ELLINGSON, J. L. E. (2006), Detection of *Mycobacterium avium* subspecies *paratuberculosis* genetic components in retail cheese curds purchased in Wisconsin and Minnesota by PCR. *Molecular and Cellular Probes*, 20, 197–202. doi: 10.1016/j.mcp.2005.12.006

CODEX ALIMENTARIUS (2003), *Codex General Standard for Cheese*, A-6-1978, Rev. 1-1999, FAO/WHO Food Standards, Food and Agriculture Organization, Rome.

COONEY, S., TIERNAN, D., JOYCE, P. and KELLY, A. L. (2000), Effect of somatic cell count and polymorphonuclear leucocyte content of milk on composition and proteolysis during ripening of Swiss-type cheese. *Journal of Dairy Research*, 67, 301–307. doi:10.1017/S0022029900004076

COULON, J. B. and RÉMOND, B. (1991), Variations in milk output and milk protein content in response to the level of energy supply to the dairy cow: a review. *Livestock Production Science*, 29, 31–47. doi:10.1016/0301-6226(91)90118-A

COULON, L. B., DELACROIX-BUCHET, A., MARTIN, B. and PIRISI, A. (2004), Relationships

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

between ruminant management and sensory characteristics of cheeses: a review. *Lait*, 84, 221–241. doi: 10.1051/lait:2004008

D'AMBROSIO, C., ARENA, S., SALZANO, A. M., RENZONE, G., LEDDA, L. and SCALONI, A. (2008), A proteomic characterization of water buffalo milk fractions describing PTM of major species and the identification of minor components involved in nutrient delivery and defense against pathogens. *Proteomics*, 8, 3657–3666. doi: 10.1002/pmic.200701148

DASEN, A., BERTHIER, F., GRAPPIN, R., WILLIAMS, A. G. and BANKS, J. (2003), Genotypic and phenotypic characterization of the dynamics of the lactic acid bacterial population of adjunct-containing Cheddar cheese manufactured from raw and microfiltered pasteurised milk. *Journal of Applied Microbiology*, 94, 595–607. doi: 10.1046/j.1365-2672.2003.01878.x

DE RHAM, O. and ANDREWS, A. T. (1982), Qualitative and quantitative determination of proteolysis in mastitic milks. *Journal of Dairy Research*, 49**,** 587–596. doi: 10.1017/S0022029900022731

DEVOLD, T. G., BROVOLD, M. J., LANGSRUD, T. and VEGARUD, G. E. (2000), Size of native and heated casein micelles, content of protein and minerals in milk from Norwegian Red Cattle – Effect of milk protein polymorphism and different feeding regimes. *International Dairy Journal*, 10, 313–323. doi:10.1016/S0958-6946(00)00073-X

DONAGHY, J. A., TOTTON, N. L. and ROWE, M. T. (2003), Evaluation of culture media for the recovery of *Mycobacterium avium* subsp *paratuberculosis* from Cheddar cheese. *Letters in Applied Microbiology*, 37, 285–291. doi: 10.1046/j.1472-765X.2003.01392.x

DONNELLY, C. W. (2004), Growth and survival of microbial pathogens in cheese. In Fox, P. F., McSweeney, P. L., Cogan, T. M. and Guinee, T. P (eds), *Cheese: Chemistry, Physics and Microbiology*, 3rd edn. London, Elsevier.

EIE, T. (1994), Meieriteknologisk anvendelse av $CO_2$ behandlet melk. Department of Food Science, Ås, Norway, Agricultural University of Norway.

EKNÆS, A. and SKEIE, S. (2006), Effect of different level of roughage availability and contrast levels of concentrate supplementation on flavour of goat milk. *Small Ruminant Research*, 66, 32–43. doi: 10.1016/j.smallrumres.2005.06.029

FARRELL, H. M., JR., JIMENEZ-FLORES, R., BLECK, G. T., BROWN, E. M., BUTLER, J. E., CREAMER, L. K., HICKS, C. L., HOLLAR, C. M., NG-KWAI-HANG, K. F. and SWAISGOOD, H. E. (2004), Nomenclature of the proteins of cows' milk – Sixth revision. *Journal of Dairy Science*, 87, 1641–1674. doi: 10.1023/A:1020659518748

FERRANTI, P., LILLA, S., CHIANESE, L. and ADDEO, F. (1999), Alternative nonallelic deletion is constitutive of ruminant alpha(s1)-casein. *Journal of Protein Chemistry*, 18, 595–602. doi: 10.1023/A:1020659518748

FOSTER, J. W. (2001), Acid stress responses of *Salmonella* and *E. coli*: Survival mechanisms, regulation, and implications for pathogenesis. *Journal of Microbiology*, 39, 89–94.

FOX, P. F., GUINEE, T. P., COGAN, T. M. and MCSWEENEY, P. L. H. (eds) (2000), *Fundamentals of Cheese Science*, Gaithersburg, MD, Aspen Publishers.

FOX, P. F., MCSWEENEY, P. L., COGAN, T. M. and GUINEE, T. P. (eds) (2004), *Cheese: Chemistry, Physics and Microbiology*, London, Elsevier.

FROHBIETER, K. A., ISMAIL, B., NIELSEN, S. S. and HAYES, K. D. (2005), Effects of *Pseudomonas fluorescens* M3/6 bacterial protease on plasmin system and plasminogen activation. *Journal of Dairy Science*, 88, 3392–3401.

GAHAN, C. G. M. and HILL, C. (1999), The relationship between acid stress responses and

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

virulence in *Salmonella typhimurium* and *Listeria monocytogenes*. *International Journal of Food Microbiology*, 50, 93–100. doi:10.1016/S0168-1605(99)00079-3

GOVINDASAMY-LUCEY, S., JAEGGI, J. J., JOHNSON, M. E., WANG, T. and LUCEY, J. A. (2007), Use of cold microfiltration retentates produced with polymeric membranes for standardization of milks for manufacture of pizza cheese. *Journal of Dairy Science*, 90, 4552–4568. doi: 10.3168/jds.2007-0128

GRANDISON, A. S. and FORD, G. D. (1986), Effects of variations in somatic-cell count on the rennet coagulation properties of milk and on the yield, composition and quality of Cheddar cheese. *Journal of Dairy Research*, 53, 645–655. doi: 10.1017/S0022029900033173

GRAPPIN, R. and BEUVIER, E. (1997), Possible implications of milk pasteurization on the manufacture and sensory quality of ripened cheese. *International Dairy Journal*, 7, 751–761. doi:10.1016/S0958-6946(98)00006-5

GRIFFITHS, M. W. (2009), *Mycobacterium paratuberculosis*. In Blackburn, C. D. W. and McClure, P. J. (eds), *Foodborne Pathogens: Hazards, Risk Analysis and Control*, 2nd edn, Cambridge, Woodhead.

GUINEE, T. P., O'BRIEN, B. and MULHOLLAND, E. O. (2007), The suitability of milk from a spring-calved dairy herd during the transition from normal to very late lactation for the manufacture of low-moisture Mozzarella cheese. *International Dairy Journal*, 17, 133–142. doi:10.1016/j.idairyj.2006.02.002

HALLÉN, E., ALLMERE, T., NÄSLUND, J., ANDRÉN, A. and LUNDÉN, A. (2007), Effect of genetic polymorphism of milk proteins on rheology of chymosin-induced milk gels. *International Dairy Journal*, 17, 791–799. doi:10.1016/j.idairyj.2006.09.011

HAN, B.-Z., MENG, Y., LI, M., YANG, Y.-X., REN, F.-Z., ZENG, Q.-K. and NOUT, M. J. R. (2007), A survey on the microbiological and chemical composition of buffalo milk in China. *Food Control*, 18, 742–746. doi:10.1016/j.foodcont.2006.03.011

HEINO, A., UUSI-RAUVA, J. and OUTINEN, M. (2008), Microfiltration of milk I: Cheese milk modification by micro- and ultrafiltration and the effect on Emmental cheese quality. *Milchwissenschaft – Milk Science International*, 63, 279–282.

HERTHNEK, D., NIELSEN, S. S., LINDBERG, A. and BOLSKE, G. (2008), A robust method for bacterial lysis and DNA purification to be used with real-time PCR for detection of *Mycobacterium avium* subsp *paratuberculosis* in milk. *Journal of Microbiological Methods*, 75, 335–340. doi: 10.1016/j.mimet.2008.07.009

HOTCHKISS, J. H., WERNER, B. G. and LEE, E. Y. C. (2006), Addition of carbon dioxide to dairy products to improve quality: a comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, 5, 158–168. doi: 10.1111/j.1541-4337.2006.00008.x

IDF (2008), The world dairy situation 2008. *IDF Bulletin*, 432.

JAROS, D., GINZINGER, W., TSCHAGER, E., LEITGEB, R. and ROHM, H. (2001), Application of oilseed feeding to reduce firmness of hard cheeses produced in the winter feeding period. *International Dairy Journal*, 11, 611–619. doi:10.1016/S0958-6946(01)00095-4

JENSEN, R. G. (2002), The composition of bovine milk lipids: January 1995 to December 2000. *Journal of Dairy Science*, 85, 295–350.

JOHANSEN, A. G., VEGARUD, G. E. and SKEIE, S. (2002), Seasonal and regional variation in the composition of whey from Norwegian Cheddar-type and Dutch-type cheeses. *International Dairy Journal*, 12, 621–629. doi:10.1016/S0958-6946(02)00054-7

JØRGENSEN, H. J., MORK, T. and RORVIK, L. M. (2005), The occurrence of *Staphylococcus aureus* on a farm with small-scale production of raw milk cheese. *Journal of Dairy Science*, 88, 3810–3817.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

KALANTZOPOULOS, G. C. (1993), Cheeses from ewes' and goats' milk. In Fox, P. F. (ed.), *Cheese: Chemistry, Physics and Microbiology. Volume 2: Major Cheese Groups*, 2nd edn. London, Chapman & Hall.

KEFFORD, B., CHRISTIAN, M. P., SUTHERLAND, B. J., MAYES, J. J. and GRAINGER, C. (1995), Seasonal influences on Cheddar cheese manufacture – Influence of diet quality and stage of lactation. *Journal of Dairy Research*, 62, 529–537. doi: 10.1017/S0022029900031228

KELLY, A. L., HUPPERTZ, T. and SHEEHAN, J. J. (2008), Pre-treatment of cheese milk: principles and developments. *Dairy Science and Technology*, 88, 549–572. doi: 10.1051/dst:2008017

KLOTZ, A., KRAUSE, I. and EINSPANIER, R. (2000), Complete sequence determination of water buffalo (*Bubalus bubalis*) beta-casein mRNA transcripts isolated from somatic milk cells. *European Food Research and Technology*, 211, 1–5. doi: 10.1007/s002170050580

KOHLMANN, K. L., NIELSEN, S. S. and LADISCH, M. R. (1991), Purification and characterization of an extracellular protease produced by *Pseudomonas fluorescens* M3/6. *Journal of Dairy Science*, 74, 4125–4136.

LEYER, G. J. and JOHNSON, E. A. (1992), Acid adaptation promotes survival of *Salmonella* spp in cheese. *Applied and Environmental Microbiology*, 58, 2075–2080.

LEYER, G. J., WANG, L. L. and JOHNSON, E. A. (1995), Acid adaptation of *Escherichia coli* O157:H7 increases survival in acidic foods. *Applied and Environmental Microbiology*, 61, 3752–3755.

LIGHTFIELD, K. D., BAER, R. J., SCHINGOETHE, D. J., KASPERSON, K. M. and BROUK, M. J. (1993), Composition and flavor of milk and Cheddar cheese higher in unsaturated fatty-acids. *Journal of Dairy Science*, 76, 1221–1232.

LINDMARK-MANSSON, H., FONDEN, R. and PETTERSSON, H. E. (2003), Composition of Swedish dairy milk. *International Dairy Journal*, 13, 409–425. doi:10.1016/S0958-6946(03)00032-3

LUCEY, J. and KELLY, J. (1994), Cheese yield. *Journal of the Society of Dairy Technology*, 47, 1–14.

MARTIN, B., BUCHIN, S. and HAUWUY, A. (2005), Influence of the botanical composition of the highland pastures on the sensory characteristics of the Beaufort cheeses. *Sciences des Aliments*, 25, 67–75.

MAYER, H. K., ORTNER, M., TSCHAGER, E. and GINZINGER, W. (1997), Composite milk protein phenotypes in relation to composition and cheesemaking properties of milk. *International Dairy Journal*, 7, 305–310. doi:10.1016/S0958-6946(97)00019-8

MCSWEENEY, P. L. H. (2007), *Cheese Problems Solved*, Cambridge, Woodhead.

MISTRY, V. V. and MAUBOIS, J.-L. (2004), Application of membrane separation technology to cheese production. In Fox, P. F., McSweeney, P. L. H., Cogan, T. M. and Guinee, T. (eds), *Cheese: Chemistry, Physics and Microbiology. Volume 1: General Aspects*, Oxford, Elsevier.

MOIOLI, B., D'ANDREA, A. and PILLA, F. (2007), Candidate genes affecting sheep and goat milk quality. *Small Ruminant Research*, 68, 179–192. doi: 10.1016/j.smallrumres.2006.09.008

MONNET, J. C., BERODIER, F. and BADOT, P. M. (2000), Characterization and localization of a cheese georegion using edaphic criteria (Jura mountains, France). *Journal of Dairy Science*, 83, 1692–1704.

MOTTAR, J. F. (1989), Effect on the quality of dairy products. In McKellar, R. C. (ed.), *Enzymes of Psychrotrophs in Raw Food*, Boca Raton, FL, CRC Press.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

NELSON, B. K., LYNCH, J. M. and BARBANO, D. M. (2004a), Impact of milk preacidification with $CO_2$ on Cheddar cheese composition and yield. *Journal of Dairy Science*, 87, 3581–3589.

NELSON, B. K., LYNCH, J. M. and BARBANO, D. M. (2004b), Impact of milk preacidification with $CO_2$ on the aging and proteolysis of Cheddar cheese. *Journal of Dairy Science*, 87, 3590–3600.

NIELSEN, S. S. and TOFT, N. (2009), A review of prevalences of paratuberculosis in farmed animals in Europe. *Preventive Veterinary Medicine*, 88, 1–14. doi: 10.1016/j.prevetmed.2008.07.003

NONI, I. D. and BATTELLI, G. (2008), Terpenes and fatty acid profiles of milk fat and 'Bitto' cheese as affected by transhumance of cows on different mountain pastures. *Food Chemistry*, 109, 299–309. doi:10.1016/j.foodchem.2007.12.033

ØSTLIE, H. M., ELIASSEN, L., FLORVAAG, A. and SKEIE, S. (2004), Phenotypic and PCR-based characterization of the microflora in Norvegia cheese during ripening. *International Journal of Food Microbiology*, 94, 287–299. doi:10.1016/j.ijfoodmicro.2004.01.012

ØSTLIE, H. M., ELIASSEN, L., FLORVAAG, A. and SKEIE, S. (2005), Phenotypic and PCR-based characterization of the microflora in Präst cheese during ripening. *International Dairy Journal*, 15, 911–920. doi:10.1016/j.idairyj.2004.08.020

PARK, Y. W., JUAREZ, M., RAMOS, M. and HAENLEIN, G. F. W. (2007), Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Research*, 68, 88–113. doi: 10.1016/j.smallrumres.2006.09.013

PULINA, G., NUDDA, A., BATTACONE, G. and CANNAS, A. (2006), Effects of nutrition on the contents of fat, protein, somatic cells, aromatic compounds, and undesirable substances in sheep milk. *Animal Feed Science and Technology*, 131, 255–291. doi:10.1016/j.anifeedsci.2006.05.023

RAJAGOPAL, M., WERNER, B. G. and HOTCHKISS, J. H. (2005), Low pressure $CO_2$ storage of raw milk: Microbiological effects. *Journal of Dairy Science*, 88, 3130–3138.

REIMERDES, E. H. and KLOSTERMEYER, H. (1976), Temperaturabhängige Veränderungen in Milch und Milchprodukten. I. Änderungen im Verhältnis Micelleneiweiss/Serumeiweiss bei der Kühlung von Milch. *Kieler Milchwirtschaftliche Forschungsberichte*, 28, 17–25.

REMEUF, F. and LENOIR, J. (1986), Relationship between the physico-chemical characteristics of goat's milk and its rennetability. *IDF Bulletin*, 68–72.

SABOYA, L. V. and MAUBOIS, J. L. (2000), Current developments of microfiltration technology in the dairy industry. *Lait*, 80, 541–553. doi: 10.1051/lait:2000144

SCHLESSER, J. E., GERDES, R., RAVISHANKAR, S., MADSEN, K., MOWBRAY, J. and TEO, A. Y. L. (2006), Survival of a five-strain cocktail of *Escherichia coli* O157:H7 during the 60-day aging period of Cheddar cheese made from unpasteurized milk. *Journal of Food Protection*, 69, 990–998.

SHAHIN, Y., HAMZAWI, L. F. and HAGGAG, H. F. (1987), Fatty acid composition of fat globule membrane neutral lipids from Egyptian buffalo, goat and cow's milk. *Food Chemistry*, 24, 11–19. doi: DOI: 10.1016/0308-8146(87)90079-3

SINGH, H. (1988), Effects of high temperatures on casein micelles. *New Zealand Journal of Dairy Sceince and Technology*, 23, 257–273.

SKEIE, S. and ARDO, Y. (2000), Influence from raw milk flora on cheese ripening studied by different treatments of milk to model cheese. *LWT – Food Science and Technology*, 33, 499–505. doi:10.1006/fstl.2000.0700

SØRHAUG, T. and STEPANIAK, L. (1991), Microbial enzymes in the spoilage of milk and

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

dairy products. In Fox, P. F. (ed.), *Food Enzymology*, London, Elsevier Applied Science.

SPAHR, U. and SCHAFROTH, K. (2001), Fate of *Mycobacterium avium* subsp *paratuberculosis* in Swiss hard and semihard cheese manufactured from raw milk. *Applied and Environmental Microbiology*, 67, 4199–4205. doi: 10.1128/AEM.67.9.4199-4205.2001

SPAHR, U. and URL, B. (1994), Behaviour of pathogenic bacteria in cheese – a synopsis of experimental data. *Bulletin of the International Dairy Federation*, 298, 2–13.

STEINSHAMN, H. and THUEN, E. (2008), White or red clover-grass silage in organic dairy milk production: Grassland productivity and milk production responses with different levels of concentrate. *Livestock Science*, 119, 202–215. doi:10.1016/j.livsci.2008.04.004

STEPHAN, R., SCHUMACHER, S., TASARA, T. and GRANT, I. R. (2007), Prevalence of *Mycobacterium avium* subspecies *paratuberculosis* in Swiss raw milk cheeses collected at the retail level. *Journal of Dairy Science*, 90, 3590–3595. doi: 10.3168/jds.2007-0015

SUHREN, G. (1989), Producer microorganisms. In McKellar, R. C. (ed.), *Enzymes of Psychrotrophs in Raw Food*, Boca Raton, FL, CRC Press.

SVANBORG, S. (2006), The fatty acid composition of milk and its effect on cheese quality. Department of Chemistry, Biotechnology and Food Science, Ås, Norway, Norwegian University of Life Sciences.

TERNSTRØM, A., LINDBERG, A. M. and MOLIN, G. (1993), Classification of the spoilage flora of raw and pasteurized bovine-milk, with special reference to *Pseudomonas* and *Bacillus*. *Journal of Applied Bacteriology*, 75, 25–34.

TINE (2007), *Annual Report 2007*. Oslo, TINE BA.

URBACH, G. (1990), Effect of feed on flavor in dairy foods. *Journal of Dairy Science*, 73, 3639–3650.

VAN DEN BERG, M. G., VAN DEN BERG, G. and VAN BOEKEL, M. (1996), Mass transfer processes involved in Gouda cheese manufacture in relation to casein and yield. *Netherlands Milk and Dairy Journal*, 50, 501–540.

VERDIER-METZ, I., COULON, J.-B., PRADEL, P., VIALLON, C. and BERDAGU, J.-L. (1998), Effect of forage conservation (hay or silage) and cow breed on the coagulation properties of milks and on the characteristics of ripened cheeses. *Journal of Dairy Research*, 65, 9–21. doi:10.1017/S0022029997002616

WALSH, C. D., GUINEE, T. P., REVILLE, W. D., HARRINGTON, D., MURPHY, J. J., O'KENNEDY, B. T. and FITZGERALD, R. J. (1998), Influence of kappa-casein genetic variant on rennet gel microstructure, cheddar cheesemaking properties and casein micelle size. *International Dairy Journal*, 8, 707–714. doi:10.1016/S0958-6946(98)00103-4

WALSTRA, P., WOUTERS, J. T. M. and GEURTS, T. J. (2006), *Dairy Science and Technology*, Boca Raton, FL, CRC/Taylor & Francis.

WEDHOLM, A., LARSEN, L. B., LINDMARK-MANSSON, H., KARLSSON, A. H. and ANDREN, A. (2006), Effect of protein composition on the cheese-making properties of milk from individual dairy cows. *Journal of Dairy Science*, 89, 3296–3305.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

# 17

# Trends in infant formulas: a dairy perspective

**R. Floris, T. Lambers, A. Alting and J. Kiers, NIZO food research B.V., The Netherlands**

IP Address: 129.132.208.100

**Abstract:** Infant food is basically specialty nutrition with highly-balanced composition aimed at mimicking breast milk, the golden standard, as closely as possible. The baby food industry nowadays offers a wide range of products trying to fulfill the changing needs of newborns and young children in their early stages of life.

Cows' milk is the starting material of most infant food products and differs in many aspects from human milk. Humanization of infant formula therefore receives great attention. Adapting the casein-whey protein ratio and increasing the $\alpha$-lactalbumin content (reducing the $\beta$-lactoglobulin content) are amongst the most obvious changes that need to be made in the final formula composition. Allergy to cows' milk is one of the possible negative effects of infant formula that can be overcome by applying hypoallergenic bovine protein hydrolysate, for which new technologies are developed to optimize taste and allergenicity.

It is realized that minor components such as oligosaccharides, nucleotides, proteins and peptides have an important function in mothers' milk and therefore technologies are developed to obtain biologically active ingredients such as bioactive peptides. Processing technologies, used to ensure the highest microbial quality of infant food, have great impact on the individual ingredients. Therefore new processing technologies such as ultra high temperature ultra short-time heating technologies and high-pressure technologies are developed and evaluated. This chapter deals with various aspects of developments of new ingredients and technologies to improve infant food.

**Key words:** human milk, humanization, casein, whey, minor components, processing, hydrolysates, allergenicity, peptides.

© Woodhead Publishing Limited, 2010

## 17.1 Introduction

Balanced nutrition provides the foundation for health. The first year of life is the most important period regarding growth, with many physical, psychological and mental changes taking place. Amongst others, digestive and immunological functions develop and nutritional needs change as a consequence. Infant food composition is adapted accordingly, resulting in a wide pallet of specifically tailored infant formula available nowadays for successive stages in life.

Infant food producers work continuously together with medical experts, nutritionists and international (non-governmental) bodies to increase the scientific knowledge of the special nutritional needs of infants and young children. Constant technological advances have enabled manufacturers to produce ever safer and healthier products to give children the best start in life (International Association of Infant Food Manufacturers, www.ifm.net).

Human breast milk is the best source of infant nutrition. The WHO recommends breastfeeding for, at least, the first 6 months of life. Infants who are deprived of this natural source for whatsoever reasons have infant formula as the only legitimate option. Human milk is the absolute gold standard for any infant formula, but establishing its exact composition is a very complex and still unfinished task. Based on scientific discoveries, many improvements have been realized since the origin of the first artificial infant milk based on cow's milk, including adapted whey/casein ratio, replacement of milk fats, and fortification with vitamins and minerals.

Over the last years, the focus seems to be shifting towards mimicking the functions of mother's milk rather than its exact composition. In Europe it is possible to add new ingredients to infant formula if their suitability for particular use has been well established by generally accepted scientific data (Koletzko *et al.*, 2002). New directions are, amongst others, reinforcement of the immune system, allergy, brain and eye development, as well as the improvement of digestibility. Targeting these needs will drive research into the discovery of suitable ingredients and technologies.

IP Address: 129.132.208.100

Starting with a brief introduction into the composition of human milk, with emphasis on its minor components, this chapter will address the current status of technological developments aimed at improving infant formula functionality, both from a nutritional as well as from a stability and safety point of view. Finally developments to produce hypoallergenic milk protein hydrolysates and bioactive peptide mixtures will be discussed.

## 17.2 Human milk

### 17.2.1 Introduction

Differences in nutrient composition of infant foods may have major effects on growth and development of neonates and, therefore, human milk acts as the gold standard for the industry. Although the complete mechanistic details remain to be identified, there is a consensus that during the postnatal period human milk,

© Woodhead Publishing Limited, 2010

amongst others, stimulates the development of the chemical and physical intestinal barrier functions, modulates the immune system and provides passive protection against pathogens. With bovine milk as the major source for infant food development, traditionally the focus for innovations was on major components. However, novel technologies (both analytical and processing technologies) have opened up novel opportunities for the industry in which important bioactivities from bovine milk are enriched or isolated to augment formulae functionality. In this respect, bioactive peptides as present in hydrolysates and/or as formed during gastrointestinal digestion show potential, as even nutritionally insignificant amounts may exert physiological effects (Meisel and FitzGerald, 2000) as further discussed below.

### 17.2.2 Human milk proteome

Novel sensitive proteomic technology developed throughout the last decade has enabled the identification of minor proteins in human milk. Especially after depletion of the major proteins secretory IgA, lactoferrin, $\alpha$-lactalbumin and HSA (Palmer *et al.*, 2006) or purification of the milk fat globular membrane proteins (Fortunato *et al.*, 2003), it was possible to obtain a more comprehensive view of the proteins secreted in human milk.

### 17.2.3 Milk fat globule membrane proteins

IP Address: 129.132.208.100

Milk fat globule membrane protein (MFGMP) constitutes a fraction of milk proteins that may display important protective effects in early infancy. Recent proteomic studies have revealed in detail the composition of the MFGMP (reviewed in Cavaletto *et al.*, 2008). Summarized, to date important bioactivities that are associated with MFGMP include antiviral, antimicrobial and immune-stimulating effects (Table 17.1). Since, generally, MFGMP is absent from infant formulae, supplementing formulae with MFGMP may be beneficial. This may simply be done by including milk fat which is generally not used in infant food formulation; however, relatively pure fractions for possible supplementation can be obtained by commercially relevant processing nowadays. It should be realized that specific bovine MFGMP components (like lactadherin) display less bioactivity than their human analogues (Kvistgaard *et al.*, 2004).

### 17.2.4 Nucleotides

Nucleotides are low-molecular-weight compounds comprising a nitrogenous base, a sugar moiety and one to three phosphate groups and belonging to the non-protein nitrogen fraction of milk. Nucleotides can be synthesized endogenously; however, they may become essential nutrients during conditions of rapid growth, malnutrition or infections. They are present in human milk as free monomeric nucleotides and nucleosides (the nucleotide metabolite form, which is the preferred form for absorption in the intestine), polymeric nucleotides

© Woodhead Publishing Limited, 2010

**Table 17.1** MFGMPs and their postulated bioactivity

| MFGMP | Activity | References |
|---|---|---|
| Mucins:<br>Muc-1<br>Muc-X | Prevention of pathogen adhesion to the gut wall; inhibition of S-fimbriated *Escherichia coli* (causing sepsis and meningitis in newborns) | Patton, (2001), Peterson *et al.* (2001) |
| Lactadherin | Inhibition of rotavirus infection | Kvistgaard *et al.* (2004) |
| Milk fat globule epidermal growth factor-VIII (MFG-E8) | Opsonization of apoptotic cells for phagocytosis (stimulates rapid clearing of possible inflammatory cellular components) | Akakura *et al.* (2004) |
| Xanthine oxidoreductase | Generation of reactive oxygen species | Martin *et al.* (2004) |

(DNA and RNA), and as part of a conjugate with other relevant compounds. In addition, metabolic precursors of nucleotides and nucleosides can be present. In their various forms nucleotides have a profound role in physiology as intermediates in energy metabolism, glycoconjugate synthesis and signal transduction. Supplementation studies with total potentially available nucleoside levels tuned to the specifications of human milk have demonstrated positive effects of ribonucleotide-supplemented infant formulae (reviewed in Gutierrez-Castrellon *et al.*, 2007). Effects include a better antibody response to common paediatric vaccinations and fewer episodes of diarrhoea. Importantly, no major risk for upper respiratory infections was identified, demonstrating that ribonucleotide supplementation has positive effects on infant health without any serious risk. A recent interesting observation is that the levels of the ribonucleotides 5′AMP, 5′GMP, 5′CMP and 5′IMP in human milk follow a circadian rhythm (Sanchez *et al.*, 2009). The rise of 5′AMP, 5′GMP and 5′UMP levels in human milk during the night is therefore suggested to be involved in a sleep-inducing effect of human milk.

IP Address: 129.132.208.100

### 17.2.5 Oligosaccharides

The microbiota of breast-fed infants is known to provide anti-infective properties and is a crucial factor for the postnatal development of the infant immune system. This is a general effect that cannot simply be correlated to a single factor, although it is commonly accepted that human milk oligosaccharides play a central role in this matter. The broad consensus is that these oligosaccharides in human milk display prebiotic effects since, to a large extent, these molecules are not digested by infants (German *et al.*, 2008). The neutral fraction of the human milk oligosaccharides appears as the most relevant factor for the development of the microbiota composition typically associated with breast-fed infants (bifidogenic effect), whereas the acidic fraction might play a role in the

© Woodhead Publishing Limited, 2010

prevention of adhesion of pathogens (Boehm and Stahl, 2007). Moreover, recently evidence was presented that human milk oligosaccharides modulate intestinal cell proliferation, differentiation and apoptosis via specialized signalling mechanisms, thus indicating that human milk oligosaccharides play a profound role in neonatal intestinal development (reviewed in Donovan, 2008).

### 17.2.6 Growth factors

Historically, growth factors have been identified to stimulate cell growth both *in vitro* and *in vivo* (hence the name growth factors). However, nowadays it is realized that these molecules have diverse functions important for the healthy development of the neonate. Importantly, of the growth factors present in human milk many exceed concentrations as present in maternal plasma, suggesting that human milk has a potent growth factor activity (Grosvenor *et al.*, 1993). Biological activities of growth factors from milk include, among others, the stimulation of growth and maturation of the gastrointestinal tract, recovery after gastrointestinal injury, control of serum insulin, and immunological protection of the GI mucosal surface (Donovan and Odle, 1994). Importantly, synergistic effects with other human milk components may occur, indicating that formula supplementation *per se* might not always be fully effective.

IP Address: 129.132.208.100

### 17.2.7 Immunoglobulins

Immunoglobulins are not transferred across the placenta to the mammalian foetus and hence infants are born with very low concentrations of serum immunoglobulins. Immunoglobulins occur in high concentration in human colostrum to provide immunological protection to the mammary gland. In addition, the absorption of immunoglobulins by infants provides passive immunity after birth, since the antibodies protect the newborn against infections (Wheeler *et al.*, 2007). The major immunoglobulins in human milk are IgA, IgG1, IgG2 and IgM. IgG1 is the main immunoglobulin type in colostrum, whereas IgM, IgA and IgG2 are present at lower concentrations. Colostrum contains approximately 100-fold higher concentrations of immunoglobulins than bovine milk and total immunoglobulin concentration decreases progressively postpartum (Marnila and Korhonen, 2002). Both IgG and IgM antibodies have multiple functions including opsonization (i.e. accentuating pathogens) to enhance phagocytosis, complement fixation for pathogen lysis, prevention of adhesion of pathogens to tissues, inhibition of microbial metabolism by blocking enzymes, agglutination of bacteria and neutralization of toxins and viruses. In contrast, secretory IgA is only involved in processes relevant to the gastro-intestinal tract, such as agglutination of bacteria, prevention of microbial adhesion to the epithelial wall and neutralization of toxins and viruses. Thus, dietary immunoglobulins may provide additional protection to the newborn.

© Woodhead Publishing Limited, 2010

### 17.2.8 Non-protein nitrogen

Compared with the milk of other species, human milk is unique in the sense that non-protein nitrogen (NPN) constitutes 20–25% of the total nitrogen. NPN components include nucleotides and their metabolites nucleosides (discussed above) (Ferreira, 2003), urea (Goedhart and Bindels, 1994), amino sugars (including sialic acid) (Nakano *et al.*, 2001), free amino acids, uric acid (Ferreira, 2003), orotic acid (Ferreira, 2003), ammonia, creatine (and creatinine) (Hülsemann *et al.*, 1987), polyamines (mainly spermine, spermidine and putrescine) (Loser, 2000), growth factors (discussed above) and amino alcohols (choline and ethanolamine) (Schuel *et al.*, 2002).

## 17.3 Humanization of infant food

### 17.3.1 Introduction

A basic formulation of infant food consists of a protein fraction, a carbohydrate fraction (lactose, starch, maltodextrin, sucrose) as energy source, and a source of fatty acids, such as vegetable oil, vitamins and minerals. As stated above, the composition of human milk clearly differs from that of other mammals such as cows, which is mostly applied as a basis for infant foods, although the milks of other mammals such as donkey, goat and mare are also known to be applied as ingredients in infant foods. Humanization of infant foods, that is the adaptation of the infant food formulation towards the composition of human milk, especially as far as its protein part is concerned, has been facilitated largely by innovations in separation technologies in the dairy industry, since the end of the last century.

IP Address: 129.132.208.100

### 17.3.2 Casein–whey protein ratio and minor components

Usage of bovine whey was first facilitated by innovations in filtration technology resulting in desalted whey preparations that enabled the formulation of products having a more human-like casein–whey protein ratio. Not only does the ratio of casein to whey protein differ from that of bovine milk (40:60 human versus 82:18 bovine), human milk also differs in lacking $\beta$-lactoglobulin, the major whey protein in bovine milk. Human milk shares this characteristic with camel milk (Kappeler *et al.*, 2003). Technologies aimed at obtaining $\alpha$-lactalbumin-enriched protein preparations were developed based on differences in heat-induced aggregation behaviour of the major whey proteins, $\alpha$-lactalbumin and $\beta$-lactoglobulin (Pearce, 1983). Especially in acidic conditions, the calcium-free form of $\alpha$-lactalbumin becomes more susceptible to heat-induced aggregation. A second, upcoming and still developing, technology to produce these kinds of preparations is (ion-exchange) chromatography. Already for years this technique has been used to produce $\alpha$-lactalbumin-enriched protein preparations. This technology is currently also applied to isolate lactoferrin and lactoperoxidase, two minor components in bovine whey, applicable to

© Woodhead Publishing Limited, 2010

humanize infant foods, as human milk is much richer in lactoferrin compared to bovine milk (Lonnerdal, 2009). It is expected that these techniques will be further developed to obtain preparations of other minor components, such as hormones and growth factors, from bovine milks or other sources (Park and Haenlein, 2006) for further fine-tuning of the compositional mimicking of human milk.

### 17.3.3 Processing and (bio)functionality

Even if the mimicking of the composition of human milk could be completed to the level of minor components, another large difference between infant foods and human milk would still exist. In contrast to human milk, infant food is processed and stored. On the other hand, during their production and storage infant foods are exposed to conditions that may affect the nutritional and functional properties of the ingredients. For example, to ensure the microbial quality of the final product, heating is applied. A major side-effect of this heating is that protein denaturation and subsequent aggregation occur (Tanford, 1968). In addition other biologically active components, such as vitamins and growth factors, may be destroyed as well. Moreover, protein modifications can occur, such as Maillard-type reactions between sugars and proteins (glycosylation) resulting in covalently linked sugar–protein complexes. In milk, the protein fraction can react with the lactose present (so-called lactosylation). An example of the effect of processing on the lactosylation of proteins in bovine milk is shown in Fig. 17.1. The observed broadening of the peaks in the reversed-phase HPLC protein separation profile is mainly caused by lactosylation of the milk proteins. It can be clearly seen that the two different types of heat treatments result in different degrees of peak broadening. This means that different degrees of lactosylation are reached during these treatments. As glycosylation of proteins may affect digestibility and bioavailability of proteins, the original

IP Address: 129.132.208.100

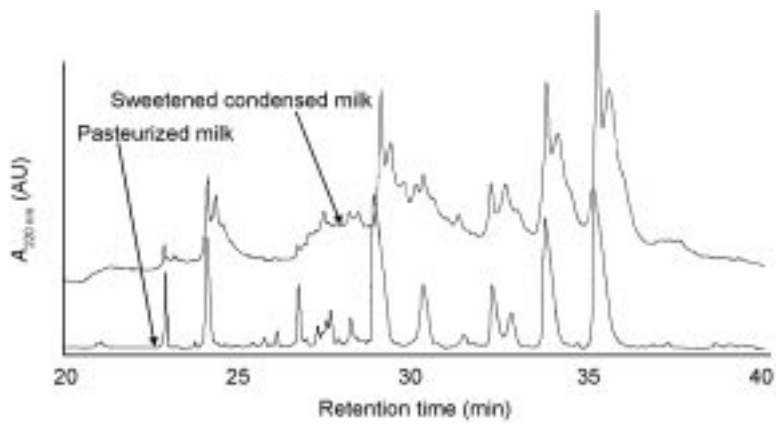



**Fig. 17.1** Comparison of RP-HPLC protein separation profiles present in differently processed milk products.

© Woodhead Publishing Limited, 2010

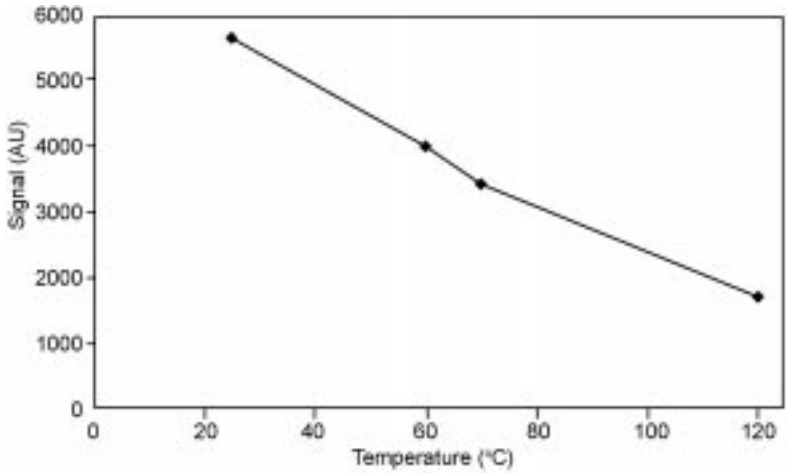



**Fig. 17.2** GlcNAc1 (N-acetyl-$\delta$-glucosamine) profile of freshly isolated cheese-whey treated at different temperatures, mimicking industrial processing (heat-loads).

functionality of these milk proteins will most likely be changed as a consequence of the processing.

In addition to protein aggregation and lactosylation, heating could also affect endogenous glycosylation of proteins, such as the complex glycosylation of $\alpha$-lactalbumin, lactoferrin and $\kappa$-casein. Figure 17.2 shows the correlation between applied heating of bovine whey and the detected levels of GlcNAc1 (N-acetyl-D-glucosamine). These kinds of complex glycosylated components are thought to play an important physiological role and their biological activity is at least in part related to the sugar moieties (Dziuba and Minkiewicz, 1996).

In conclusion, during processing of infant foods and especially during the applied heating steps, reactions will occur that affect the integrity of ingredients. As a result it may be expected that these modifications will affect the functionality of the ingredients. For example, it is generally known that lactosylation will decrease the number of available lysine residues and may affect digestibility, bioavailability and possibly allergenicity. It is expected that denaturation/aggregation reactions will affect the biological function of the modified component, since this biological activity is mostly related to the conformation of the native molecules (e.g lactoferrin and lactoperoxidase are inactive when denatured).

IP Address: 129.132.208.100

### 17.3.4 New preserving technologies, innovative steam injection

In order to assure the safety of infant foods, micro-organisms have to be inactivated. This means that processing steps will always be required. The most commonly applied technique to achieve this is heat treatment. As described, however, the drawback is that heating may impair the quality of foods, causing unacceptable loss of functionality, such as destruction of vitamins, protein

© Woodhead Publishing Limited, 2010

denaturation and formation of off-flavours. An additional problem, which has emerged in recent years, is spoilage of foods by very heat-resistant bacterial spores originating from raw materials. So-called minimal processing techniques like high-pressure treatment and pulsed electric fields (PEF) appear to be insufficient to inactivate bacterial spores (De Jong and Heesch, 1998; Pol *et al.*, 2001). The application of ultra-high temperatures (>150ºC) and ultra-short residence times (<0.2 s) might offer new opportunities with regard to microbial inactivation while preserving product quality (Van Asselt *et al.*, 2008). This new time–temperature profile allows significant inactivation of heat-resistant spores (46 decimal reductions), while preserving the (bio)functionality of important ingredients to a large extent.

### 17.3.5 Enhancing function versus mimicking composition

Processing mostly affects functionality of ingredients as stated above. Therefore, it may be questioned if 'complete' humanization of infant foods can be reached by completely mimicking the composition of human milk. Infant foods will always differ from human milk in the degree of processing and this will affect the functionality of the ingredients in infant foods. Therefore, a practical approach is to aim for equivalent functionality rather than for an equal composition. The focus here is to retain or add a specific biological functionality. A recent example is the application of a mixture of galacto/fructo-oligosaccharides (GOS/FOS) to modulate the infants' intestinal microflora towards one that is rich in bifidobacteria as observed in breast-fed infants (Bakker-Zierikzee *et al.*, 2005). This bifidogenic effect of human milk has been recognized for many years, and naturally present complex (branched) oligosaccharides have been recognized as the responsible components (Boehm *et al.*, 2007).

GOS is another innovative example of how the dairy industry develops ingredients from whey. GOS is an oligosaccharide resulting from the enzymatic action of $\beta$-galactosidase on lactose. FOS is obtained from chicory extract. Since the majority of oligosaccharides from bovine milk have a different composition from those present in human milk and levels may be too low to provide for a sufficient biological effect (Gopal and Gill, 2000; Tao *et al.*, 2008), other ingredients that mimic the functionality of human milk oligosaccharides can be applied.

Another very illustrative example is the addition of probiotic bacteria to infant formula. Rather than stimulating beneficial gut bacteria through oligosaccharide supplementation, this concept deals with the actual addition of beneficial health-promoting bacteria. Probiotics have benefits in steering the immune response as has recently been demonstrated (van Baarlen *et al.*, 2009). Until recently it was thought that human milk did not contain bacteria, except for carry-over from the skin, and therefore the addition of bacteria would fall within this category of mimicking function rather than composition. However, recent findings may warrant a review of this statement (Lara-Villoslada *et al.*, 2007).

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

## 17.4 Towards optimized composition: analytical tools and models

With human milk as a reference, development of functional infant formulae requires sensitive analytical methodologies. Nowadays several specialized techniques aiming at target identification, quantification of bioactives and quality control are available, enabling continuous innovation in infant food. For example, methods for quantification of lactoferrin, oligosaccharide profiling (Ninonuevo *et al.*, 2006), comparative and quantitative proteomics (Goshe and Smith, 2003), phospholipid and nucleotide quantification have been designed.

## 17.5 Infant food and allergenicity

### 17.5.1 General introduction

For the production of infant foods, bovine milk has been the major source. Above it was discussed that although, to the largest extent, the composition of human milk can be mimicked, differences will always exist because of processing-induced changes in (bio)functionality. In addition, it has been realized for many decades that cow's milk allergy (CMA) is a major issue in the production of infant formula. Allergy, best described as an inappropriate response against innocuous antigens, is, in most cases, caused by proteins. Allergy to cow's milk proteins is the most common allergy in infancy. Cow's milk is usually the first food antigen introduced in an infant's diet, and therefore cow's milk allergy is often the first presentation of the atopic constitution of an individual (Sampson, 2004). Although CMA is often transient in childhood, children with CMA are at greater risk of developing other atopic disorders, including atopic dermatitis, allergic asthma and allergic rhinitis. Although tolerance to cow's milk develops in more than 85% of infants before the age of 3 years (Bock, 1987; Host and Halken, 1990; Jakobsson and Lindberg, 1979), a small number retain their allergy. Cow's milk allergy in adults is rare but usually severe. Recently it was shown that in these patients both caseins and whey proteins play a negative role (Lam *et al.*, 2008). In fact the major infant formulae producers have been focusing on reduction of allergenicity almost from the start of their business.

In order to be successful in the development of these formulae, it is vital to know what exactly is causing cow's milk allergy. The protein fraction of milk consists of caseins ($\alpha_{s1}$-, $\alpha_{s2}$-, $\beta$- and $\kappa$-casein) and whey proteins ($\alpha$-lactalbumin and $\beta$-lactoglobulin being the most abundant). The IgE response during allergy is generally directed to all major proteins (van Beresteijn *et al.*, 1995) as shown in Fig. 17.3.

The casein fraction of cow's milk protein appears to be more allergenic than whey proteins in both children and adults (Stoger and Wuthrich, 1993; Szabo and Eigenmann, 2000) and from the individual caseins $\alpha_{s1}$-casein was shown to be the most potent allergen and $\kappa$-casein the least allergenic (Bernard *et al.*, 1998).

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

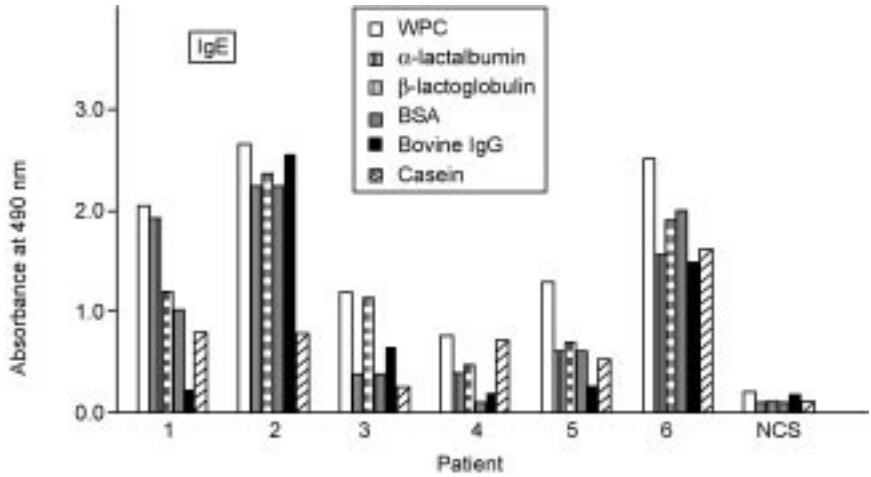



**Fig. 17.3** Serum levels of specific IgE antibodies in six patients against casein, bovine $\alpha$-lactalbumin, bovine $\beta$-lactoglobulin, BSA and bovine immunoglobulins.

On the other hand, $\beta$-lactoglobulin, the major whey protein, is also claimed to be one of the major milk allergens (Wal, 2004). This protein is absent from human milk and relatively resistant to proteolytic attack and gastric acid. Moreover, during the first months in life this protein is able to cross the intestinal barrier intact in infants.

Under normal conditions, after digestion in the stomach, peptides and amino acids enter the duodenum, where further degradation takes place before absorption via the mucosal membrane, following which they serve as nutrients. Oligopeptides below eight amino acids (smaller than 3000 Da) are not presented or recognized by immune cells and are therefore immunologically inert (Suri *et al.*, 2006). However, a small part of food proteins are not fully degraded and can elicit immune responses after absorption (Untersmayr and Jensen-Jarolim, 2006). Especially in young children, due to the immaturity of the digestive system (and the higher pH in the stomach), the amount of partly degraded proteins absorbed will be higher (Lonnerdal, 2003). This increases the chance of the presence of immunologically active milk protein fragments that can be recognized as foreign by the immune system, resulting in the induction of immune responses. The best infant nutrition is human milk, because in addition to having many factors (e.g. immunoglobulins, lysozyme, lactoferrin, lactoperoxidase, growth factors) that provide passive immunity and protection to the newborn, its protein is not allergenic (Lonnerdal, 2003).

IP Address: 129.132.208.100

### 17.5.2 Technologies to produce hypoallergenic infant formula

The infant food industry has developed various methods and processing technologies to reduce allergenicity of cow's milk proteins, which are generally used in infant formula. Enzymatic hydrolysis of bovine milk proteins, aimed at

© Woodhead Publishing Limited, 2010

eliminating allergenic epitopes, is the most effective strategy that has led to successful hypoallergenic infant food products (Host and Halken, 2004). The optimal hydrolysis with respect to immunological properties and nutritional value is, however, unknown and strongly depends on the type of proteins present in the starting material (e.g. whey or casein-based). The main challenge, when using hydrolysis, is maintaining palatability (bitterness is the most prevalent problem here), nutritional properties and functional properties of the original proteins (Host and Halken, 2004).

That enzymatic hydrolysis of cow's milk protein in infant formulas is an effective strategy to reduce overall allergenicity was, amongst others, demonstrated by a study from van Beresteijn *et al.*, in which the serum of patients allergic to cow's milk was shown to have antibodies to fractions of cow's milk and the residual antigenicity of hydrolysates was lower than that of intact proteins (van Beresteijn *et al.*, 1995). However, immunoreactive epitopes could still be detected in all tested, commercially available products. Even a 3000 Da hydrolysate was shown to contain IgE epitopes, although this particular hydrolysate did not elicit allergic reactions in experimental animals (van Beresteijn *et al.*, 1994) suggesting that, in this case, physiological digestion reactions could completely eliminate residual epitopes.

A combination of selective hydrolysis (using a lactococcal extracellular envelope protease) of caseins in a milk protein mixture followed by ultra-filtration (cutoff values between 3 and 30 kDa) resulted in casein hydrolysates free of protein fragments that were cross-reactive with whey-protein-specific IgE (Alting *et al.*, 1998). In addition, casein itself was shown to be degraded in such a way that cross-reactive casein-specific IgE antibody-binding sites were eliminated, showing that the epitopes in caseins were completely destroyed. This hydrolysate out-performed commercially available hydrolysates in terms of hypoallergenicity.

IP Address: 129.132.208.100

Thus, for hydrolysis aiming at allergy reduction the choice of the enzyme or enzyme combinations is very important, even if only low molecular weight fractions are taken as protein material with reduced allergenicity (Wroblewska *et al.*, 2007). For example, pepsin, the protease that naturally occurs in the stomach, was shown to be hardly effective in reducing the antigenicity of cow's milk protein hydrolysates in combination with other proteases.

The goal for these hydrolysis processes is always to eliminate the epitopes of the milk proteins. The exact nature of these epitopes, however, is unknown, which hampers rational approaches. At least for $\beta$-lactoglobulin, a systematic approach was used to get more insight into the parts of the molecule that were recognized by T-cells from patients with cow's milk allergy, and seven peptides were identified to which the T-cells from these patients responded, producing high levels of Il-5 (Inoue *et al.*, 2001).

In conclusion, milk protein hydrolysis is an effective strategy to produce hypoallergenic formula; however, the production of an effective hydrolysate may still be further optimized: specific destruction of epitopes is still an important target.

© Woodhead Publishing Limited, 2010

### 17.5.3 High pressure

A new approach to eliminate allergenic milk protein epitopes more efficiently is the use of physical treatments to denature the proteins before enzymatic treatment, thereby providing better substrates for the proteases, expected to result in more efficient hydrolysis (Bonomi *et al.*, 2000). High pressure is a rather new technology that nowadays is finding its way into industrial practice, although production-scale application remains a challenge. Under certain temperature and high pressure conditions $\beta$-lactoglobulin forms transient conformations with regions of the hydrophobic core unfolded. These new protein conformations present new target peptide bonds for enzymatic hydrolysis, as compared with the native protein (Bonomi *et al.*, 2003).

For $\beta$-lactoglobulin it was shown that high-pressure treatment in the presence of the enzymes trypsin and chymotrypsin indeed leads to a reduction of IgG binding (Chicón *et al.*, 2008). This is in line with the finding that pressure-induced changes of globular whey proteins have an effect on the proteolysis (Belloque *et al.*, 2007). However, peptides containing IgE epitopes were still formed under the conditions used. It was therefore suggested that screening for the right conditions (and enzyme combinations) would lead to the development of hydrolysates with a further reduced allergenicity. Indeed such an approach was followed and was shown to be successful, i.e. screening of different enzymes under various conditions led to the development of hypoallergenic bovine whey protein hydrolysates (Peñas *et al.*, 2006). This shows the potential of combining physical technologies, such as high hydrostatic pressure, and enzyme technologies for further development of hypoallergenic milk protein hydrolysates for the infant food industry. This can be of great interest for the industry, since in commercially available hypoallergenic milk formulas potentially allergenic material can still be detected, such as residual antigenic $\beta$-lactoglobulin, even in extensively hydrolysed products (Rosendal and Barkholt, 2000).

IP Address: 129.132.208.100

## 17.6 Other beneficial properties of milk protein hydrolysates

### 17.6.1 Increased rate of uptake

Besides reduction of formula allergenicity, milk protein hydrolysates are also used in infant food to improve overall digestibility of the formula and provide a better nutrient uptake. In fact, it has been shown that the uptake of amino acids in the form of peptides (di- and tri-peptides) occurs more readily than that of free amino acids in man (Silk *et al.*, 1982). This is another important reason to use hydrolysed milk proteins in infant formulas.

### 17.6.2 Bioactive peptides

In recent years it has been recognized that milk proteins are a rich source of biologically active peptides. These peptides, encrypted in the milk protein, are

© Woodhead Publishing Limited, 2010

inactive within the sequence of the parent protein, but can be released by enzymatic hydrolysis. Upon ingestion these peptides may affect major physiological systems, namely the digestive, immune and nervous systems, depending on their amino acid sequences. These peptides may also act locally, e.g. in the gut to modulate the intestinal microbiota. Especially since particular peptides are potent and may exert physiological effects in nutritionally insignificant amounts, dietary peptides have a large potential to promote health effects (Korhonen and Pihlanto, 2006). An overview is given in Table 17.2 of specific groups of bioactive peptides that can be derived from milk proteins.

The antimicrobial peptides are of particular interest, since these peptides may play a role in maintaining a healthy gut microflora. In this respect lactoferricin (a potent antimicrobial lactoferrin-derived peptide) has been detected along the gastro-intestinal tract of mice (Kuwata *et al.*, 1998), indicating the potential *in vivo* activity. In order to obtain efficacy data to support a new or improved infant formula *in vivo* nutritional intervention trials are essential. There is a need to reduce the number of these (animal) trials in discovery programmes for ethical and financial reasons. Up to now predictive computer models aiming to circumvent and/or reduce the number of these *in vivo* experiments are rare or too complex, with many non-measurable adjustable parameters. Recently a basic physico-chemical computer model for a first quantitative interpretation of results obtained from *in vivo* intestinal experiments with bacteria was developed (de Jong *et al.*, 2007). This new modelling approach was validated with results obtained from gut infection studies *in vivo* and could be used to test the hypothesis that the growth rate of *E. coli* in the colon of the rat and man is different.

There is a large number of milk-derived peptides that could display antibacterial or antiviral properties (Floris *et al.*, 2003) and this potential of milk

IP Address: 129.132.208.100

**Table 17.2** Overview of bioactive peptides and their potential effects that can be derived from the various milk proteins by means of enzymatic hydrolysis

| Protein source | Effect | Name |
|---|---|---|
| $\alpha$-, $\beta$-caseins | Mineral binding | Phosphopeptides |
| $\kappa$-casein | Opioid antagonist | Casoxin |
| $\alpha$-, $\beta$-, $\kappa$-caseins | Opioid agonist | Casomorphins |
| $\alpha$-, $\beta$-, $\kappa$-caseins | Immune modulation | Immunopeptides |
| $\alpha$-, $\kappa$-caseins | Antimicrobial | Isracidin/casocidin-I/ casecidin |
| $\kappa$-casein | Antithrombotic | Casoplatelins |
| $\alpha$-, $\beta$-caseins | Antihypertensive | Casokinins |
| $\alpha$-lactalbumin | Opioid agonist, ACE inhibition | $\alpha$-lactorphin |
| $\beta$-lactoglobulin | Non-opioid stimulatory effect on ileum, ACE inhibition | $\beta$-lactorphin |
| | Ileum contraction | $\beta$-lactotensin |
| BSA | Opioid activity | Serorphin |
| | Ileum contraction, ACE inhibition | Albutensin A |
| Lactoferrin | Bactericidal activity | Lactoferricin |

© Woodhead Publishing Limited, 2010

protein hydrolysates to display biological effects is not yet exploited by the infant food industry. Therefore peptides offer large opportunities for further innovations and development of new products. In these product development trajectories the challenge will be to balance the hypoallergenic properties of a hydrolysate with the presence of the desired bioactive peptides. Importantly, since many hydrolysates are already utilized in infant foods, establishing the presence of possible physiological active peptides in these hydrolysates may be a first step in identifying novel functionalities in available infant formula.

### 17.6.3 Tolerogenic peptides

Finally, peptides can play a role in inducing oral tolerance to milk proteins, defined as the specific suppression of cellular and/or humoral immune response to an antigen by prior administration of the antigen via the oral route (Faria and Weiner, 2005). If oral tolerance fails, food allergy occurs. In animal models, oral tolerance against food proteins has been achieved with the use of moderately hydrolysed proteins as inducers. Induction of such a specific oral tolerance by tolerogenic peptides (peptides that induce tolerance) derived from food proteins is seen as a powerful measure to drag the immune system towards tolerance instead of sensitization. For $\beta$-lactoglobulin it has now been shown that specific peptides derived from this protein indeed could be used to make mice tolerant against $\beta$-lactoglobulin. Tolerogenic peptides could be a very important tool for the infant food industry to help overcome milk protein allergy in children.

IP Address: 129.132.208.100

### 17.6.4 Reduction of bitterness

Bitterness is a major drawback of hydrolysed food products and especially infant food formulae. Caseins are notoriously bitter when hydrolysed (Lemieux and Simard, 1992) and therefore casein-derived hydrolysates have not been applied widely in the food industry unless intensively hydrolysed. This bitter off-taste represents a technical problem that has not been adequately solved by the food industry (Pawlett and Bruce, 1996). There are some general masking approaches to reduce bitterness, such as addition of artificial sweeteners, addition of flavours, addition of bitter blockers (e.g. adenosine monophosphate) and processing measures (e.g. ultrafiltration, column chromatography) to remove bitter peptides. However, none of these approaches reduces bitterness sufficiently, and moreover in many cases, due to strict regulation, these measures are not always allowed in infant food preparation.

Recently, however, a novel approach was followed using a proline-specific enzyme that resulted in debittering of casein hydrolysates (Edens *et al.*, 2005). Such new approaches are very promising and could lead to the development of a new generation of hydrolysates for the infant food industry by combining low allergenicity with reduced bitterness.

© Woodhead Publishing Limited, 2010

### 17.6.5 Use of other protein sources: rice proteins

A widely used approach to help children with cow's milk allergy is to switch to hydrolysates based on other proteins. The most common approach here is to prescribe soy formula. However, allergic reactions to soy formulas are known amongst atopic children (Bruno *et al.*, 1997). It was therefore investigated whether rice hydrolysate formula could be used for children with cow's milk and soy allergy. Indeed, children with these allergies could tolerate such a hydrolysate, indicating that rice protein is a promising food source for children with multiple food-induced allergies (Fiocchi *et al.*, 2003). In common practice this novel source, however, needs to be further evaluated.

## 17.7 Conclusions

Human milk is the best source of nutrition for infants. Many attempts have been and are being undertaken in order to fully unravel its composition and functionalities. This is largely driving the development and improvement of infant formula by industry (reviewed by Nasirpour *et al.*, 2006), resulting in foods with specific nutritional properties targeted at specific age categories and/or need states. One of today's hottest scientific interests is the understanding of the impact of early infant feeding on the metabolic state in later life, especially in relation to (childhood) obesity. As a result of such increasing scientific understanding, the incorporation of speciality ingredients (not rarely non-dairy) such as pre- and probiotics, and long chain, polyunsaturated fatty acids (mainly derived from fish oil), known to be important in visual and brain development amongst others, has been and will be driving infant formula innovations. Protecting these usually sensitive components during processing, storage and food preparation is a major challenge and requires the continuous optimization of technologies such as micro-encapsulation and mild (heat) processing.

Of major future importance will be the allergy epidemic that is foreseen. Two major ingredient classes play an important role. The need for carefully selected and designed protein hydrolysates will continue to increase, not only guaranteeing hypo-allergenicity, but comprising additional bioactive functionalities because of carefully designed peptide patterns. Secondly, pre- and probiotics have been identified as a means to affect immune priming. Clinical trials are being published, but true scientific mechanistic understanding is lacking. Scientific research in this area will greatly expand in the coming years and will eventually show the potential of these ingredients.

Industry cannot restrict its efforts to these nutritional innovations, since infant foods require usually a long shelf-life, which demands significant processing to ensure food safety. This has a very serious impact not only on their nutritional quality but also their physical stability, and the organoleptic properties are affected, which creates the need for innovative technological solutions. Typically the control of sensoric properties of protein hydrolysates as well as off-flavour management will be ongoing areas of attention. A rather

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

undeveloped area would be the understanding of the behaviour of foods in the infantile digestive tract. As an example, textural and flavour properties influence food liking and intake, as well as stomach emptying and satiety. In times of heavy competition and demanding consumers, addressing these latter aspects could make the additional difference.

## 17.8 References

AKAKURA, S., SINGH, S., SPATARO, M., AKAKURA, R., KIM, J. I., ALBERT, M. L. and BIRGE, R. B. (2004) The opsonin MFG-E8 is a ligand for the alphavbeta5 integrin and triggers DOCK180-dependent Rac1 activation for the phagocytosis of apoptotic cells. *Exp Cell Res*, 292, 403–416.

ALTING, A., MEIJER, R. J. G. M. and VAN BERESTEIJN, E. C. H. (1998) Selective hydrolysis of milk proteins to facilitate the elimination of the ABBOS epitope of bovine serum albumin and other immunoreactive epitopes. *J Food Prot*, 61, 1007–1012.

BAKKER-ZIERIKZEE, A. M., ALLES, M. S., KNOL, J., KOK, F. J., TOLBOOM, J. J. and BINDELS, J. G. (2005) Effects of infant formula containing a mixture of galacto- and fructo-oligosaccharides or viable Bifidobacterium animalis on the intestinal microflora during the first 4 months of life. *Br J Nutr*, 94, 783–790.

BELLOQUE, J., CHICÓN, R. and LOPEZ-FANDINO, R. (2007) Unfolding and refolding of beta-lactoglobulin subjected to high hydrostatic pressure at different pH values and temperatures and its influence on proteolysis. *J Agric Food Chem*, 55, 5282–5288.

BERNARD, H., CREMINON, C., YVON, M. and WAL, J. M. (1998) Specificity of the human IgE response to the different purified caseins in allergy to cow's milk proteins. *Int Arch Allergy Immunol*, 115, 235–244.

BOCK, S. A. (1987) Prospective appraisal of complaints of adverse reactions to foods in children during the first 3 years of life. *Pediatrics*, 79, 683–688.

BOEHM, G. and STAHL, B. (2007) Oligosaccharides from milk. *J Nutr*, 137, 847S–849S.

BOEHM, G., BEERMANN, C., GEORGI, G. and STAHL, B. (2007) Infant formulas. In Y. H. Hui (ed.), *Handbook of Food Products Manufacturing*, Hoboken, NJ: Wiley Interscience, 677–714.

BONOMI, F., JAMETTI, S., RAMUSSEN, P., RESTAIN, P. and ROVERE, P. (2000) Advances in the combined application of enzymatic and physical treatments for reducing food allergenicity. *High Pressure Res*, 19, 175–181.

BONOMI, F., FIOCCHI, A., FROKIAER, H., GAIASCHI, A., IAMETTI, S., POIESI, C., RASMUSSEN, P., RESTANI, P. and ROVERE, P. (2003) Reduction of immunoreactivity of bovine beta-lactoglobulin upon combined physical and proteolytic treatment. *J Dairy Res*, 70, 51–59.

BRUNO, G., GIAMPIETRO, P. G., DEL GUERCIO, M. J., *ET AL.* (1997) Soy allergy is not common in atopic children: a multicenter study. *Pediatr Allergy Immunol*, 8, 190–193.

CAVALETTO, M., GIUFFRIDA, M. G. and CONTI, A. (2008) Milk fat globule membrane components–a proteomic approach. *Adv Exp Med Biol*, 606, 129–141.

CHICÓN, R., BELLOQUE, J., ALONSO, E., MARTÍN-ÁLVAREZ, P. J. and LÓPEZ-FANDINO, R. (2008) Hydrolysis under high hydrostatic pressure as a means to reduce the binding of $\beta$-lactoglobulin to immunoglobulin E form human sera. *J Food Prot*, 71, 1453–1459.

DE JONG, P. and HEESCH, E. J. M. (1998) Review, effect of pulsed electric fields on the quality of food products. *Milchwissenschaft*, 53, 4–8.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

DE JONG, P., VISSERS, M. M., VAN DER MEER, R. and BOVEE-OUDENHOVEN, I. M. (2007) In silico model as a tool for interpretation of intestinal infection studies. *Appl Environ Microbiol*, 73, 508–515.

DONOVAN, S. M. (2008) Human milk oligosaccharides – the plot thickens. *Br J Nutr*, 1–3.

DONOVAN, S. M. and ODLE, J. (1994) Growth factors in milk as mediators of infant development. *Annu Rev Nutr*, 14, 147–167.

DZIUBA, J. and MINKIEWICZ, P. (1996) Influence of glycosylation on micelle-stabilizing ability and biological properties of C-terminal fragments of cow's κ-casein. *Int Dairy J*, 6, 1017–1044.

EDENS, L., DEKKER, P., VAN DER HOEVEN, R., DEEN, F., DE ROOS, A. and FLORIS, R. (2005) Extracellular prolyl endoprotease from Aspergillus niger and its use in the debittering of protein hydrolysates. *J Agric Food Chem*, 53, 7950–7957.

FARIA, A. M. and WEINER, H. L. (2005) Oral tolerance. *Immunol Rev*, 206, 232–259.

FERREIRA, I. M. (2003) Quantification of non-protein nitrogen components of infant formulae and follow-up milks: comparison with cows' and human milk. *Br J Nutr*, 90, 127–133.

FIOCCHI, A., TRAVAINI, M., D'AURIA, E., BANDERALI, G., BERNARDO, L. and RIVA, E. (2003) Tolerance to a rice hydrolysate formula in children allergic to cow's milk and soy. *Clin Exp Allergy*, 33, 1576–1580.

FLORIS, R., RECIO, I., BERKHOUT, B. and VISSER, S. (2003) Antibacterial and antiviral effects of milk proteins and derivatives thereof. *Curr Pharm Des*, 9, 1257–1275.

FORTUNATO, D., GIUFFRIDA, M. G., CAVALETTO, M., GAROFFO, L. P., DELLAVALLE, G., NAPOLITANO, L., GIUNTA, C., FABRIS, C., BERTINO, E., COSCIA, A. and CONTI, A. (2003) Structural proteome of human colostral fat globule membrane proteins. *Proteomics*, 3, 897–905.

GERMAN, J. B., FREEMAN, S. L., LEBRILLA, C. B. and MILLS, D. A. (2008) Human milk oligosaccharides: evolution, structures and bioselectivity as substrates for intestinal bacteria. *Nestlé Nutr Workshop Ser Pediatr Program*, 62, 205–218; discussion 218–222.

GOEDHART, A. and BINDELS J. G. (1994) The composition of human milk as a model for the design of infant formulas: recent findings and possible applications. *Nutr Res Rev*, 7, 1–23.

GOPAL, P. K. and GILL, H. S. (2000) Oligosaccharides and glycoconjugates in bovine milk and colostrum. *Br J Nutr*, 84 Suppl 1, S69–S74.

GOSHE, M. B. and SMITH, R. D. (2003) Stable isotope-coded proteomic mass spectrometry. *Curr Opin Biotechnol*, 14, 101–109.

GROSVENOR, C. E., PICCIANO, M. F. and BAUMRUCKER, C. R. (1993) Hormones and growth factors in milk. *Endocr Rev*, 14, 710–728.

GUTIERREZ-CASTRELLON, P., MORA-MAGANA, I., DIAZ-GARCIA, L., JIMENEZ-GUTIERREZ, C., RAMIREZ-MAYANS, J. and SOLOMON-SANTIBANEZ, G. A. (2007) Immune response to nucleotide-supplemented infant formulae: systematic review and meta-analysis. *Br J Nutr*, 98 Suppl 1, S64–S67.

HOST, A. and HALKEN, S. (1990) A prospective study of cow milk allergy in Danish infants during the first 3 years of life. Clinical course in relation to clinical and immunological type of hypersensitivity reaction. *Allergy*, 45, 587–596.

HOST, A. and HALKEN, S. (2004) Hypoallergenic formulas – when, to whom and how long: after more than 15 years we know the right indication! *Allergy*, 59 Suppl 78, 45–52.

HÜLSEMANN, J., MANZ, F., WEMBER, T. and SCHÖCH, G. (1987) Administration of creatine and creatinine with breast milk and infant milk preparations. *Klin Pädiatr*, 199, 292–295.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

INOUE, R., MATSUSHITA, S., KANEKO, H., SHINODA, S., SAKAGUCHI, H., NISHIMURA, Y. and KONDO, N. (2001) Identification of beta-lactoglobulin-derived peptides and class II HLA molecules recognized by T cells from patients with milk allergy. *Clin Exp Allergy*, 31, 1126–1134.

JAKOBSSON, I. and LINDBERG, T. (1979) A prospective study of cow's milk protein intolerance in Swedish infants. *Acta Paediatr Scand*, 68, 853–859.

KAPPELER, S. R., FARAH, Z. and PUHAN, Z. (2003) 5′-flanking regions of camel milk genes are highly similar to homologue regions of other species and can be divided into two distinct groups. *J Dairy Sci*, 86, 498–508.

KOLETZKO, B., ASHWELL, M., BECK, B., BRONNER, A. and MATHIOUDAKIS, B. (2002) Characterisation of infant food modifications in the European Union. *Ann Nutr Metab*, 46, 231–242.

KORHONEN, H. and PIHLANTO, A. (2006) Bioactive peptides: production and functionality. *Int Dairy J*, 16, 945–960.

KUWATA, H., YIP, T. T., YAMAUCHI, K., TERAGUCHI, S., HAYASAWA, H., TOMITA, M. and HUTCHENS, T. W. (1998) The survival of ingested lactoferrin in the gastrointestinal tract of adult mice. *Biochem J*, 334(2), 321–323.

KVISTGAARD, A. S., PALLESEN, L. T., ARIAS, C. F., LOPEZ, S., PETERSEN, T. E., HEEGAARD, C. W. and RASMUSSEN, J. T. (2004) Inhibitory effects of human and bovine milk constituents on rotavirus infections. *J Dairy Sci*, 87, 4088–4096.

LAM, H. Y., VAN HOFFEN, E., MICHELSEN, A., GUIKERS, K., VAN DER TAS, C. H., BRUIJNZEEL-KOOMEN, C. A. and KNULST, A. C. (2008) Cow's milk allergy in adults is rare but severe: both casein and whey proteins are involved. *Clin Exp Allergy*, 38, 995–1002.

LARA-VILLOSLADA, F., OLIVARES, M., SIERRA, S., RODRIGUEZ, J. M., BOZA, J. and XAUS, J. (2007) Beneficial effects of probiotic bacteria isolated from breast milk. *Br J Nutr*, 98 Suppl 1, S96–S100.

LEMIEUX, L. and SIMARD, R. E. (1992) Bitter flavour in dairy products. II A review of bitter peptides form caseins: their formation, isolation and identification, structure masking and inhibition. *Lait*, 72, 335–382.

LONNERDAL, B. (2003) Nutritional and physiologic significance of human milk proteins. *Am J Clin Nutr*, 77, 1537S–1543S.

LONNERDAL, B. (2009) Nutritional roles of lactoferrin. *Curr Opin Clin Nutr Metab Care*, 12, 293–297.

LOSER, C. (2000) Polyamines in human and animal milk. *Br J Nutr*, 84 Suppl 1, S55–S58.

MARNILA, P. and KORHONEN, H. (2002) Immunoglobulins. In H. Roginski, J.W. Fuquay and P. F. Fox (eds), *Encyclopedia of Dairy Sciences*, London: Academic Press, 1950–1956.

MARTIN, H. M., HANCOCK, J. T., SALISBURY, V. and HARRISON, R. (2004) Role of xanthine oxidoreductase as an antimicrobial agent. *Infect Immun*, 72, 4933–4939.

MEISEL, H. and FITZGERALD, R. J. (2000) Opioid peptides encrypted in intact milk protein sequences. *Br J Nutr*, 84 Suppl 1, S27–S31.

NAKANO, T., SUGAWARA, M. and KAWAKAMI, H. (2001) Sialic acid in human milk: composition and functions. *Acta Paediatr Taiwan*, 42, 11–17.

NASIRPOUR, A., SCHER, J. and DESOBRY, S. (2006) Baby foods: formulations and interactions (a review). *Crit Rev Food Sci Nutr*, 46, 665–681.

NINONUEVO, M. R., PARK, Y., YIN, H., ZHANG, J., WARD, R. E., CLOWERS, B. H., GERMAN, J. B., FREEMAN, S. L., KILLEEN, K., GRIMM, R. and LEBRILLA, C. B. (2006) A strategy for annotating the human milk glycome. *J Agric Food Chem*, 54, 7471–7480.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

PALMER, D. J., KELLY, V. C., SMIT, A. M., KUY, S., KNIGHT, C. G. and COOPER, G. J. (2006) Human colostrum: identification of minor proteins in the aqueous phase by proteomics. *Proteomics*, 6, 2208–2216.

PARK, Y. W. and HAENLEIN, G. F. W. (2006) *Handbook of Milk of Non-bovine Mammals*. Oxford and Ames, IA: Blackwell Publishing.

PATTON, S. (2001) MUC1 and MUC-X, epithelial mucins of breast and milk. *Adv Exp Med Biol*, 501, 35–45.

PAWLETT, D. and BRUCE, G. (1996) Debittering of protein hydrolysates. In T. Godfrey and S. West (eds), *Industrial Enzymology*, 2nd edn. London: Macmillan, 179–186.

PEARCE, R. J. (1983) Thermal seperation of beta-lactoglubulin and alpha-lactalbumin in bovine Cheddar cheese whey. *Aust J Dairy Technol*, 38, 144–149.

PEÑAS, E., SNEL, H., FLORIS, R., PRÉSTAMO, G. and GOMEZ, R. (2006) High pressure can reduce the antigenicity of bovine whey protein hydrolysates. *Int Dairy J*, 16, 969–975.

PETERSON, J. A., SCALLAN, C. D., CERIANI, R. L. and HAMOSH, M. (2001) Structural and functional aspects of three major glycoproteins of the human milk fat globule membrane. *Adv Exp Med Biol*, 501, 179–187.

POL, I. E., VAN ARENDONK, W. G., MASTWIJK, H. C., KROMMER, J., SMID, E. J. and MOEZELAAR, R. (2001) Sensitivities of germinating spores and carvacrol-adapted vegetative cells and spores of Bacillus cereus to nisin and pulsed-electric-field treatment. *Appl Environ Microbiol*, 67, 1693–1699.

ROSENDAL, A. and BARKHOLT, V. (2000) Detection of potentially allergenic material in 12 hydrolyzed milk formulas. *J Dairy Sci*, 83, 2200–2210.

SAMPSON, H. A. (2004) Update on food allergy. *J Allergy Clin Immunol*, 113, 805–819; discussion 820.

SANCHEZ, C. L., CUBERO, J., SANCHEZ, J., CHANCLON, B., RIVERO, M., RODRIGUEZ, A. B. and BARRIGA, C. (2009) The possible role of human milk nucleotides as sleep inducers. *Nutr Neurosci*, 12, 2–8.

SCHUEL, H., BURKMAN, L. J., LIPPES, J., CRICKARD, K., FORESTER, E., PIOMELLI, D. and GIUFFRIDA, A. (2002) N-Acylethanolamines in human reproductive fluids. *Chem Phys Lipids*, 121, 211–227.

SILK, D. B., HEGARTY, J. E., FAIRCLOUGH, P. D. and CLARK, M. L. (1982) Characterization and nutritional significance of peptide transport in man. *Ann Nutr Metab*, 26, 337–352.

STOGER, P. and WUTHRICH, B. (1993) Type I allergy to cow milk proteins in adults. A retrospective study of 34 adult milk- and cheese-allergic patients. *Int Arch Allergy Immunol*, 102, 399–407.

SURI, A., LOVITCH, S. B. and UNANUE, E. R. (2006) The wide diversity and complexity of peptides bound to class II MHC molecules. *Curr Opin Immunol*, 18, 70–77.

SZABO, I. and EIGENMANN, P. A. (2000) Allergenicity of major cow's milk and peanut proteins determined by IgE and IgG immunoblotting. *Allergy*, 55, 42–49.

TANFORD, C. (1968) *Physical Chemistry of Macromolecules*. New York: John Wiley & Sons.

TAO, N., DEPETERS, E. J., FREEMAN, S., GERMAN, J. B., GRIMM, R. and LEBRILLA, C. B. (2008) Bovine milk glycome. *J Dairy Sci*, 91, 3768–3778.

UNTERSMAYR, E. and JENSEN-JAROLIM, E. (2006) Mechanisms of type I food allergy. *Pharmacol Ther*, 112, 787–798.

VAN ASSELT, A., SWEERE, A. P. J., ROLLEMA, H. S. and DE JONG, P. (2008) Extreme high-temperature treatment of milk with respect to plasmin inactivation. *Int Dairy J*, 18, 531–538.

VAN BAARLEN, P., TROOST, F. J., VAN HEMERT, S., VAN DER MEER, C., DE VOS, W. M., DE GROOT,

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

P. J., HOOIVELD, G. J., BRUMMER, R. J. and KLEEREBEZEM, M. (2009) Differential NF-kappaB pathways induction by Lactobacillus plantarum in the duodenum of healthy humans correlating with immune tolerance. *Proc Natl Acad Sci USA*, 106, 2371–2376.

VAN BERESTEIJN, E. C. H., PEETERS, R. A., KAPER, J., MEIJER, R. J. G. M., ROBBEN, A. J. P. M. and SCHMIDT, D. G. (1994) Molecular mass distribution, immunological properties and nutritive value of whey protein hydrolysates. *J Food Prot*, 57, 619–625.

VAN BERESTEIJN, E. C., MEIJER, R. J. and SCHMIDT, D. G. (1995) Residual antigenicity of hypoallergenic infant formulas and the occurrence of milk-specific IgE antibodies in patients with clinical allergy. *J Allergy Clin Immunol*, 96, 365–374.

WAL, J. M. (2004) Bovine milk allergenicity. *Ann Allergy Asthma Immunol*, 93, S2–S11.

WHEELER, T. T., HODGKINSON, A. J., PROSSER, C. G. and DAVIS, S. R. (2007) Immune components of colostrum and milk – a historical perspective. *J Mammary Gland Biol Neoplasia*, 12, 237–247.

WROBLEWSKA, B., JEDRYCHOWSKI, L. and FARJAN, M. (2007) The allergenicity of a low molecular fraction of cow milk protein hydrolysates. *Milchwissenschaft*, 62, 375–379.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

# 18

# Applications of milk components in products other than foods

**J.-L. Audic and B. Chaufer, Université de Rennes, France**

**Abstract:** This chapter deals with applications of milk in products other than foods. Non-food uses of casein and whey proteins are first described, focusing on their uses in films and coatings. The following section discusses whey fermentation and applications for lactose. The final section reviews applications for milk fat.

**Key words:** non-food applications, milk proteins, casein, whey proteins, lactose.

IP Address: 129.132.208.100

## 18.1 Introduction

Milk components are mainly used in the food industry, but numerous applications in products other than foods have also been reported. Milk can be used as a raw material in the manufacture of plastic goods [1], textile fibres [2], glues [3] and paints, for example, or as a substrate for fermentation to produce a wide range of compounds such as organic acids, solvents or biogas. Some of the applications of milk in non-food products have been recognized for a long time [3] and on occasion patented. New applications have recently been proposed on account of the specific properties of milk and milk components [4]. These aim to add value to dairy effluents that would otherwise be disposed of as waste, generating high biological oxygen demand, and have opened up new markets for milk and milk co-products. Some of the non-food applications described in this review concern individual components of milk that must be recovered by chemical and/or physical means. In other cases, applications

© Woodhead Publishing Limited, 2010

concern diluted milk, or milk fractions that contain various components together; this greatly simplifies the separation and purification steps. Value-added products can thus be obtained by direct fermentation of dairy wastes or dairy co-products such as whey.

The first part of the present review deals with applications of milk proteins, i.e. casein, the main milk protein, and whey proteins, in products other than foods. Particular attention is paid to the thermoplastic and film-forming properties of milk proteins and their use in the manufacture of biodegradable materials. Milk proteins can be regarded as natural polymers that can be used in the manufacture of plastic goods, protective films and coatings. Casein and whey proteins are not only studied for their applications in biodegradable materials: they also find niche applications in other domains. Most historical applications involve casein. The protein has been used since the beginning of the twentieth century in the manufacture of glues, rigid plastics and textile fibres. Fewer technical applications have been reported in the past for whey proteins. Recently, however, their use in cosmetics and pharmacology, for instance, has been reported.

The applications of lactose in the non-food area are also reviewed with reference to its functional properties. It is used not only as a raw material in pharmaceuticals, but also as a substrate for fermentation, or for synthesis of different derivatives like lactulose or lactitol. Non-food uses of whey, a mixture of lactose, whey proteins, salts and vitamins, which is a co-product of cheese making and the casein industry, are highlighted. Indeed, fermentation processes generally involve the direct use of whey or whey permeate without further purification. The small number of non-food applications for milk fat are also covered.

IP Address: 129.132.208.100

## 18.2 Non-food uses of major components of milk: a short review

All non-food applications cited for individual milk components and for milk whey are listed in Table 18.1.

### 18.2.1 Milk proteins

*Biodegradable materials from milk proteins*

Proteins in general, including casein or whey protein, can be considered as thermoplastic heteropolymers based on 20 amino acid monomers that have different side groups attached to the central carbon. Thus, several applications deal with the use of milk protein as a natural polymer that would replace synthetic petroleum polymers in material applications. Due to the low frequency of secondary structures, milk proteins are considered as random coil polypeptides with a high degree of molecular flexibility able to form inter- and intra-specific interactions like hydrogen or van der Waals bonds. This

© Woodhead Publishing Limited, 2010

**Table 18.1** Technical applications of the different milk components

| Milk component | Property | Applications | Remarks |
|---|---|---|---|
| Milk proteins: casein | Film-forming ability | Paint | Still used |
| | Adhesion | Ink | Still used |
| | Technical properties | Paper | Still used |
| | | Packaging | To be developed |
| | | Textile coating | Historical |
| | Processability | Water-based glues | Historical |
| | Bond strength | | |
| | Strength | Rigid plastics | Historical |
| | Good mechanical properties | Fibres | Historical |
| | | Coatings/films | To be developed |
| Milk proteins: whey proteins | Specific properties of lactoglobulin, lactalbumin, lactoferrin, etc. | Cosmetology | In use |
| | | Pharmacology | To be developed |
| | Film-forming ability | Coatings/films | To be developed |
| | | Paper coating | In use |
| | Solubility | Surfactants | In use |
| | Viscosity | Creams/shampoos | In use |
| | Emulsifying properties and water sorption | Cosmetology | Few applications |
| | | Pharmacology | Few applications |
| Lactose | Reactive sites | Chemical feedstock | New |
| | Disaccharide | Lactulose, lactitol, lactobionic acid, etc. | In development |
| | Carbon source for fermentation | See whey | See whey |
| Whey | Substrate for fermentation | Solvents (ethanol, butanol) | In use |
| | | Biogas (methane) | In use |
| | Lactose as C source | Organic acids (lactic, citric, acetic, lactobionic, itaconic, etc.) | In use |
| | Whey proteins as N source | | |
| | | Polysaccharides (xanthan, pullulan, dextran, gellan, etc.) | In use |
| | | Vitamins | Few applications |
| | | Oils | Few applications |
| | | Yeasts/biomass | In use |
| Milk fat | Emollient properties | Pharmacology | Few applications |
| | | Cosmetology | Few applications |
| | Hydrophobicity | Protective films/coatings | Few applications |
| | Hydrolysis into mono- or disaccharides and fatty acids | Emulsifiers | In use |

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

confers upon these proteins good film-forming ability. Thus, milk protein-based films and coatings have been widely studied during the last 20 years [5–8] in order to find some new biodegradable materials able to partially or totally replace synthetic polymers obtained from non-renewable resources.

In recent years, the interest in the development of films and coatings for food made from renewable natural biopolymers has grown for various reasons, such as food quality, convenience and more proactive attitudes towards reducing the environmental impact of packaging wastes. In fact, because of their biodegradability, bioplastics are especially used in disposable items like packaging films, coatings, plastic bags and containers for fruits or vegetables. The market for biodegradable polymers has shown strong growth during the last five years, but there are still only very few commercial-scale production plants. The major classes of biopolymers actually commercially available are starch, starch-based blends, polylactic acid (PLA) and aliphatic–aromatic co-polyesters. However, the market volumes for biopolymers remain very low compared to standard petrochemical-based plastics. In 2005, the global biodegradable plastics market tonnage is estimated at about 95,000 tonnes compared with 28,000 tonnes in 2000. In 2010, market tonnage of biodegradable plastics is forecast to reach more than 214,000 tonnes [9]. Protein materials based on casein or whey proteins are not (yet) produced commercially or serve niche markets. However, milk proteins should find numerous niche applications considering their specific properties (to be detailed later) and the global biodegradable plastics annual growth rate of about 17–20% [9, 10]. European Bioplastics projects a further growth of bio-based polymers in the EU to 2–4 millions tonnes in 2020. Half of this total consists of compostable products; the other half is durables.

IP Address: 129.132.208.100

Even if most biopolymers are biodegradable, they are also generally obtained from non-renewable resources. Thus, most of the aromatic–aliphatic polyesters like polybutylene terephthalate (PBT), polytrimethylene terephthalate (PTT), polybutylene succinate (PBS) or some biodegradable polyurethanes are nowadays produced from petrochemical feedstock [11]. Then, compared to these commercially available materials (nowadays the only ones present on the market), protein-based films are not only biodegradable but also present the main advantage of being obtained from renewable resources.

Whey proteins and casein are known to give transparent films with very good resistance to oxygen, aroma and lipid transfer. Because milk protein-based films are initially brittle and sensible to water (with high water solubility and water vapour permeability), their mechanical properties as well as their water resistance need to be improved. Mechanical properties are controlled through the addition of plasticizers. Plasticizers used in the literature are generally polyols such as glycerol, which is certainly the most used. Through the addition of 30–50% w/w of plasticizer it is possible to obtain protein films with improved mechanical properties compatible with applications in packaging. Oxygen permeabilities and tensile properties of milk protein-based films are compared to conventional synthetic films in Table 18.2.

© Woodhead Publishing Limited, 2010

**Table 18.2** Comparison of oxygen permeabilities (OP) and tensile properties (tensile strength, TS, and elongation at break, $\epsilon_b$) of protein-based films and synthetic films

| Film[a] | Test conditions | TS (MPa) | $\epsilon_b$ (%) | OP[b] | Refs |
|---|---|---|---|---|---|
| NaCAS/Gly (0%) | 50% RH, RT | 57 | 4 | | [12] |
| NaCAS/Gly (28%) | 50% RH, RT | 6.2 | 63.2 | | [12] |
| NaCAS/Gly (50%) | 50% RH, RT | 2.4 | 91.6 | | [12] |
| WPI/Gly (50%) | 55/76% RH, 23ºC | 5.8 | 22.7 | | [7, 13] |
| WPC/Gly (50%) | 55/74% RH, 23ºC | 3.5 | 20.8 | | [7, 13] |
| $CO_2$CAS/Gly (30%) | 50% RH, RT | 1.2–3 | 50.2–74.2 | | [14] |
| $CO_2$CAS/Gly (30%) | 50% RH, 23ºC | 5 | 56 | 144 | [15] |
| CaCAS/Gly (30%) | 50% RH, 23ºC | 7 | 66 | 86 | [15] |
| WPI/Gly (30%) | 50% RH, 23ºC | 13.9 | 30.8 | 76.1 | [16] |
| CaCAS/Gly (30%) | 50% RH, RT | 1.6–1.9 | 66.6–76 | | [14] |
| WPI/Gly (33.3%) | 50% RH, 25ºC | | | 11–16 | [17] |
| WPC coated PE[c] | <60% RH, 23ºC | | | <30 | [17] |
| WPC coated PP[c] | <60% RH, 23ºC | | | <30 | [17] |
| LDPE | 50% RH, 23ºC | 13 | 500 | 1870 | [18] |
| HDPE | 50% RH, 23ºC | 26 | 300 | 427 | [18] |
| EVOH | 50% RH, 23ºC | | | 0.2 | [19] |
| PVDC-based films[d] | 50% RH, 23ºC | 26.2 | 400 | 0.33 | |

[a] Abbreviations: WPI, whey protein isolate; WPC, whey protein concentrate; NaCAS, sodium caseinate; CaCAS, calcium caseinate; $CO_2$CAS, $CO_2$ precipitated casein; LDPE, low density polyethylene; HDPE, high density polyethylene; EVOH, ethylene vinyl alcohol; PVDC, polyvinylidene chloride; PE, polyethylene; PP, polypropylene; Gly, glycerol. The value between brackets refers to glycerol content in % w/w.

[b] mL.$\mu$m/m$^2$.day.kPa.

[c] WPC is plasticized with 33.3% glycerol.

[d] From commercial data sheet, Saranex 450®, Dow Chemical Company.

IP Address: 129.132.208.100

Nevertheless, tensile strength and elongation at break are still below the values obtained for conventional plastic films. In order to improve cohesion, tensile strength, rigidity and barrier properties of protein films, proteins can be crosslinked with bifunctional compounds like dialdehydes or diisocyanates, for example, or by physical treatment like $\gamma$-irradiation [20–23]. The occurrence of covalent bridges between protein chains allows a water-insoluble network to be achieved, increasing cohesion between protein chains. Moreover, enzymatic crosslinking treatments with transglutaminases or peroxidases can also be applied to stabilize protein films [24–26]. Divalent ions like $Ca^{2+}$ were tested in the same way but they lead to rather little effect on protein film cohesion and solubility in water [24]. The more commonly used covalent crosslinking agents described in recent papers are glutaraldehyde, glyceraldehyde, formaldehyde, gossypol, tannic and lactic acids [27].

In order to increase water vapour resistance and mechanical properties, milk protein films can be formulated with fatty acids [13] or waxes [28]. Saturated fatty acids greatly limit water vapour permeability, and waxes are commonly used as hydrophobic substances in coatings and films to increase the barrier properties against water. By providing gloss, waxes are also known to enhance

© Woodhead Publishing Limited, 2010

the visual appeal of food products. It was also demonstrated that waxes can partially replace hydrophilic plasticizers required in protein films and coatings [28].

Another important application for milk protein-based films and coatings is their use as barrier materials in packaging applications. Oxygen-barrier layers in food packaging materials typically consist of synthetic barrier polymers including ethylene vinyl alcohol (EVOH) copolymers, polyvinylidene chloride (PVDC), polyethylene terephthalate (PET) or polyamides (PA) [17]. The low oxygen permeability of whey protein films as well as casein-based films in addition to good gloss and mechanical properties make milk proteins potentially useful for the manufacture of films or coatings with improved $O_2/CO_2$ barrier properties [15, 17, 29]. In Table 18.2, oxygen permeability and tensile properties of milk protein films are compared to conventional synthetic polymers used in packaging applications. For example, oxygen-barrier coatings based on whey proteins can be formed on common plastic films such as polyethylene (PE) or polypropylene (PP) [17]. The resulting whey protein-coated plastic films have excellent $O_2$-barrier properties (OP inferior to 30 mL.$\mu$m/m$^2$.day.kPa), comparable to those of synthetic films used in such applications. Whey protein coatings could replace synthetic oxygen-barrier layers, including EVOH, PVDC and PA, to provide new paths for the use of whey proteins and to improve recyclability of packaging films. Generally, an oxygen-barrier polymer is defined as a polymer having an OP value of lower than about 40 mL.$\mu$m/m$^2$.day.kPa at 23ºC [18].

In order to give added value to protein films obtained from relatively expensive resources (more expensive than synthetic petro-polymers), niche applications can be developed in particular areas such as in active packaging. Through the incorporation of antimicrobial agents it is possible to reduce the level of foodborne pathogens and to control undesirable microorganisms in foods during storage and distribution [30, 31].

IP Address: 129.132.208.100

As well as films and packages, casein can be used in other material applications, such as in the manufacture of rigid plastic goods [3, 32] (Table 18.1). Because of the presence of side groups on the protein backbone that can be chemically or enzymatically modified, casein is cured by bifunctional compounds like formaldehyde in most applications where rigidity is required [33–35]. In the well-known example of rigid plastic patented under the trade name Galalith® ('milk stone'), casein and fillers are mixed in water before extrusion into plastic goods, which are crosslinked with formaldehyde. The resulting three-dimensional network makes the final product more resistant to water and increases its mechanical strength. Such casein plastics are still used today in buttons or buckles, but such articles are becoming more and more limited compared to articles made from cheaper synthetic plastics, which also exhibit better thermo-mechanical properties.

*Other non-food uses of milk proteins*

Casein was also used in the manufacture of glues and many different formulations have been published or patented [3, 36–38] (Table 18.1): these glues are

© Woodhead Publishing Limited, 2010

generally composed of casein and an alkali as the two major constituents mixed in water. Water resistance is obtained through crosslinking with a third ingredient like lime or copper chloride. Casein- or milk-based glues were described by the ancient Egyptians and in the Middle Ages but nowadays casein glues are used in only a few applications [38] (Table 18.1), including in labelling adhesives, in the bottling industry, in bonding paper or in pressure-sensitive adhesives [36, 39]. Due to their amphipathic nature, proteins are often used as emulsifiers or stabilizers. Casein is thus claimed to be employed in the formulation of concrete or cements [32, 40, 41] as well as in soaps [42], dishwashing liquids or various cosmetics [43, 44]. In water-based paints [45–48], the soluble caseinate is used to bind pigments. Casein is also used as a stabilizer and emulsifier in oil or latex paint [39, 49, 50]. Because of the presence of both polar and apolar groups, casein also presents good affinity for pigments, good ink binding properties and adhesion to various substrates. Thus, the major milk protein is also used in the paper industry as a sizing agent for the manufacture of high quality glazed papers [41, 51]. The coating is cured with formaldehyde or dialdehydes to confer water resistance to the paper. In a similar way, casein is also employed as a coating agent in the textile industry [3, 52–55] to promote resistance to abrasion, to enhance pigment bindings and water resistance, or to confer antistatic properties to textile fibres. Textile fibres can also be prepared from casein [2, 56]: an alkali-solution of casein is spun into a coagulation bath containing acid and inorganic salts. Once again, water resistance is obtained by chemical crosslinking of the spun fibres with formaldehyde [57–59]. Casein fibres were mostly produced during the Second World War under the trade names of Aralac®, Fibrolane® or Lannital® to replace wool or cotton, but they since have been supplanted by more competitive synthetic fibres [1].

In some aspects, whey proteins present physical and functional properties similar to those of casein: adhesion, emulsifying properties, water sorption and gel forming properties and film-forming properties. Thus, most of the non-traditional uses proposed for whey proteins are identical to those of casein and are listed in Table 18.1. These include manufacture of films and coatings [16, 60–63], paper coating [64], and emulsifiers [65] in cream and shampoos. Nevertheless, some particular whey proteins like $\beta$-lactoglobulin or $\alpha$-lactalbumin present their own specific properties: such proteins are extracted from whey and then included 'as is' in cosmetics or drugs [43, 65, 66].

IP Address: 129.132.208.100

### 18.2.2 Lactose and whey fermentations

Annual global production of whey, the main co-product of the cheese-making industry, is estimated to be 72 million tons, which means that about 1.2 million tons of lactose is transferred into whey annually [67]. Large quantities of lactose and whey are available on the market. Though there have been several new technological developments in the transformation of milk whey to other useful products [67, 68], utilization or disposal of whey remains a major problem in the dairy industry.

© Woodhead Publishing Limited, 2010

Lactose, the characteristic carbohydrate of bovine milk, is a disaccharide of glucose and galactose with several reactive sites such as the reducing group of glucose or hydroxyl groups that can be chemically or enzymatically modified. Thus, lactose is used as a raw material in the chemical industry [67, 69–73] (Table 18.1). Some lactose derivatives of great interest are lactulose [67, 74], used in the pharmaceutical industry as an effective drug against acute and chronic constipation [67], lactitol [75, 76], used in the manufacture of surface modifiers and emulsifiers, and lactobionic acid [75] used as a gel-firming agent. Galacto-oligosaccharides [77] are also obtained from lactose by a transgalactosylation reaction. Like lactulose, these oligosaccharides are known to promote the growth of *Bifidobacterium* species in the colon. Lactosyl urea and some esters, halogenated derivatives and anhydro derivatives have also been synthesized from lactose [71, 78, 79]. Lactose was also employed as a chain extender in the synthesis of polyurethanes [80]. In the same way, some other attempts have been made to valorize lactose from whey permeate obtained by ultrafiltration techniques (deproteinated whey). Such permeate is used in the formulation of phenol-formaldehyde resins such as plywood adhesives [81].

Nevertheless, most of the non-food applications proposed for lactose concern direct fermentation of whole whey [69, 71, 82–84], which is an aqueous solution of lactose (4.9–5.1%), soluble proteins (0.9–1%), ashes (0.5–0.7%), fat (0.1–0.3%) and lactic acid (0–0.2%) [85]. Fermentation is a profitable way to obtain value-added products and to reduce dairy waste streams at the same time. Such products are listed in Table 18.1. In most processes dealing with whole whey fermentation, lactose needs to be first hydrolysed and an extra nitrogen source (for example, through soluble protein hydrolysis) must be added to achieve good growth and productivity of microorganisms [68, 86].

IP Address: 129.132.208.100

All the products obtained from the processes developed worldwide can be classified into three main categories [87]. First, low value products obtained in high volume: this is the case for methane [88] (and other biogas [89]) or ethanol [42, 90–92]. The methanogenic process [93] based on anaerobic fermentation of cheese whey is able to produce biogas composed of about 50% $CH_4$: theoretically 1 kg of lactose yields 0.75 $m^3$ biogas [69]. Recently, cheese whey was used to produce hydrogen gas which can potentially be used as a fuel: the yield corresponded to the production of 2.9 litre of $H_2$ per litre of cheese whey. Several distilleries also produce ethanol from whey that can further be used as a fuel or to produce vinegar or acetic acids. The second category of products resulting from whey fermentation are products of intermediate value obtained in high volume. In this category we find organic acids [94] like acetic [95], propionic [96], lactic [97–99], lactobionic [100], citric [101] or gluconic [102] acids that are generally further used in speciality chemicals. Lactic acid is used to produce biodegradable polylactic acid polymers and recent interest in biodegradable polymers has led to enhanced demand for the acid. Food products and yeasts are also included in this category. The third category comprises high value fermentation products obtained in low volume like antibiotics, healthcare products, enzymes, vitamins [68, 69] or various exopolysaccharides [103–108].

© Woodhead Publishing Limited, 2010

Among the most valuable products, exopolysaccharides like xanthan gum present a potentially large market in a broad range of industries, such as in foods, toiletries, oil recovery, cosmetics, paints, etc. [78, 108]. The demand for xanthan gum has increased steadily every year and is estimated to grow continuously at an annual rate of 5–10% [108]. Increasing market price and demand suggest that commercial production of xanthan gum from cheese whey (or lactose) is an innovative process that should be developed in the future. Among all the products obtained from whey or lactose fermentation, another is oil that can be produced from whey fermentation by certain yeasts in order to replace vegetable oils [109].

### 18.2.3 Milk fat

Few non-food specific uses of milk fat in products other than foods have been reported (Table 18.1). Milk fat includes mono-, di- and triglycerides, fatty acids, cholesterol, milk fat globule membrane and phospholipids. Due to its emollient properties, it can be used in the manufacture of soaps, creams or shampoos [110, 111]. Some patents report the use of 0.5–10% hydroxylated triglycerides (Cremerol HMG, Amerchol Corp.) in the manufacture of soaps [111]. Milk lipid liposomes and ceramides are used in skin care creams to keep the skin hydrated. Sphingolipids and lauric acid present in milk fat exhibit antimicrobial properties that could be used in pharmacological applications. New emulsifiers made of mono- and diglycerides can also be produced through milk fat hydrolysis. Milk fat has also been proposed for the manufacture of hydrophobic protective films and coatings [110, 112]. Compared to other edible lipid films used in such applications as wax-based films, the milk fat fraction is significantly more viscous, less elastic and more easily deformed. Thus, edible materials based on milk fat are softer and offer much less resistance to deformation than other waxes generally used in packaging applications. This viscoelastic behaviour means that milk fat-based materials would be more flexible and less likely to crack [113].

IP Address: 129.132.208.100

## 18.3 Conclusions

This review constitutes a survey of actual and potential non-food applications of milk components. It particularly focuses on some general applications in regard to the specific properties of each component.

Stickiness and viscosity as well as film-forming ability explain why caseinate solutions are used in the manufacture of glues, coatings (paper coatings, sizing agents), films and biomaterials. Casein is also an additive acting as an emulsifying agent in various products like paints, concrete and cements or dishwashing liquids. Like caseins, soluble proteins are used in the manufacture of films and coatings and as an additive in several formulations considering their emulsifying and gel-forming properties. Single soluble proteins also find specific applications in cosmetology and pharmacology. Among the non-food applications of

© Woodhead Publishing Limited, 2010

milk proteins (casein, soluble protein), coatings, films and packaging are of high interest in the field of biodegradable polymers.

Lactose is used as a raw material for the manufacture of derivatives like lactitol, lactulose or lactobionic acid. Nevertheless, most of the non-food uses deal with fermentation processes of lactose providing a wide range of valuable products such as biogas (methane), alcohols, organic acids, baker's yeast, vitamins and exopolysaccharides. Fermentation of (hydrolysed) lactose from whey permeates or whole whey is an alternative way to contribute to the reduction of dairy waste streams.

Concerning milk fat, actually only few non-food applications have been developed such as milk fat-based emollients or fatty acid production, but the commercial potential seems to be promising. New markets need to be developed, as dairy and food products tend to reduce their fat content.

Non-food valorizations proposed in this paper for milk should also be applied to dairy wastes and co-products in order to reduce waste streams.

## 18.4 References


1. SOUTHWARD, C.R. (1989), Uses of casein and caseinates, in *Developments in Dairy Chemistry – 4. Functional Milk Proteins*, ed. P.F. Fox, London: Elsevier Applied Science, pp. 173–243.
2. FERETTI, A. (1937), Production of the artificial wool called Lannital from casein. *Ind. Textile*, **54**: 446–447.
3. TAGUE, E.L. (1926), Casein. Its Preparation, Chemistry and Technical Utilization, New York: Van Nostrand Co.
4. AUDIC, J.L. and B. CHAUFER (2005), Influence of plasticizers and crosslinking on the properties of biodegradable films made from sodium caseinate. *Eur. Polym. J.*, **41**(8): 1934–1942.
5. ARVANITOYANNIS, I., E. PSOMIADOU, and A. NAKAYAMA (1996), Edible films made from sodium caseinate, starches, sugars and glycerol. *Carbohyd. Polym.*, **31**: 179–192.
6. AUDIC, J.L., F. FOURCADE, and B. CHAUFER (2007), Biodegradable material obtained from renewable resource: Plasticized sodium caseinate films, in *Thermodynamics, Solubility and Environmental Issues*, ed. T. Letcher, Oxford: Elsevier, pp. 336–345.
7. CHEN, H. (1995), Functional properties and applications of edible films made of milk proteins. *J. Dairy Sci.*, **78**: 2563–2583.
8. CHICK, J. and Z. USTUNOL (1998), Mechanical and barrier properties of lactic acid and rennet precipitated casein-based edible films. *J. Food Sci.*, **63**(6): 1024–1027.
9. PLATT, D. (2006), *Biodegradable Polymers. Market Report*. Shawbury, Shrewsbury, UK: Smithers Rapra Ltd.
10. STEWART, R. (2007), Biopolymers greater consumer awareness drives growth of bio-based-resins market. *Plastics Engineering* (Brookfield, CT), **63**(6): 24–30.
11. PATEL, M.K. and M. CRANK (2007), Projections for the production of bulk volume bio-based polymers in Europe and environmental implications. *J. Biobased Mater. Bio.*, **1**: 437–453.
12. PEREDA, M., M.I. ARANGUREN, and N.E. MARCOVICH (2008), Characterization of chitosan/caseinate films. *J. Appl. Polym. Sci.*, **107**(2): 1080–1090.


IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

13. ERDOHAN, Z.O. and K.N. TURHAN (2005), Barrier and mechanical properties of methylcellulose-whey protein films. *Packag. Technol. Sci.*, **18**(6): 295–302.
14. TOMASULA, P.M., *et al.* (1998), Properties of films made from $CO_2$-precipitated casein. *J. Agric. Food Chem.*, **46**(11): 4470–4474.
15. TOMASULA, P.M., W.C. YEE, and N. PARRIS (2003), Oxygen permeability of films made from CO2-precipitated casein and modified casein. *J. Agric. Food Chem.*, **51**(3): 634–639.
16. MCHUGH, T.H. and J.M. KROCHTA (1994), Sorbitol-vs glycerol-plasticized whey protein edible films: Integrated oxygen permeability and tensile property evaluation. *J. Agric. Food Chem.*, **42**: 841–845.
17. HONG, S.-I. and J.M. KROCHTA (2006), Oxygen barrier performance of whey-protein-coated plastic films as affected by temperature, relative humidity, base film and protein type. *J. Food Eng.*, **77**(3): 739–745.
18. SALAME, M. (1986), *The Wiley Encyclopedia of Packaging Technology*, ed. M. Baker, New York: Wiley.
19. BYUN, Y.J., *et al.* (2007), Physical and chemical properties of gamma-irradiated EVOH film. *Radiat. Phys. Chem.*, **76**(6): 974–981.
20. LACROIX, M., *et al.* (2002), Use of gamma-irradiation to produce films from whey, casein and soya proteins: Structure and functional characteristics. *Radiat. Phys. Chem.*, **63**(3–6): 827–832.
21. CIESLA, K., S. SALMIERI, and M. LACROIX (2006), Gamma-irradiation influence on the structure and properties of calcium caseinate-whey protein isolate based films. Part 1. Radiation effect on the structure of protein gels and films. *J. Agric. Food Chem.*, **54**(17): 6374–6384.
22. RESSOUANY, M., C. VACHON, and M. LACROIX (1998), Irradiation dose and calcium effect on the mechanical properties of cross-linked caseinate films. *J. Agric. Food Chem.*, **46**(4): 1618–1623.
23. RESSOUANY, M., C. VACHON, and M. LACROIX (2000), Microbial resistance of caseinate films crosslinked by gamma irradiation. *J. Dairy Res.*, **67**(1): 119–124.
24. GALIETTA, G., *et al.* (1997), Mechanical and thermomechanical properties of films based on whey proteins as affected by plasticizer and crosslinking agents. *J. Dairy Sci.*, **81**: 3123–3130.
25. MOTOKI, M., *et al.* (1987), $\alpha_{s1}$-casein film prepared using transglutaminase. *Agric. Biol. Chem.*, **51**: 993–996.
26. MOTOKI, M. and K. SEGURO (1998), Transglutaminase and its use for food processing. *Trends Food Sci. Technol.*, **9**: 204–210.
27. MARQUIÉ, C., C. AYMARD, J.L. CUQ, and S. GUILBERT (1995), Biodegradable packaging made from cottonseed flour: Formation and improvement by chemical treatment with gossypol, formaldehyde and glutaraldehyde. *J. Agric. Food Chem.*, **43**: 2762–2766.
28. MIN, S.C., T. JANJARASSKUL, and J.M. KROCHTA (2009), Tensile and moisture barrier properties of whey protein-beeswax layered composite films. *J. Sci. Food Agric.*, **89**(2): 251–257.
29. SCHOU, M., *et al.* (2005), Properties of edible sodium caseinate films and their application as food wrapping. *LWT – Food Sci. Technol.*, **38**(6): 605–610.
30. KRISTO, E., K.P. KOUTSOUMANIS, and C.G. BILIADERIS (2007), Thermal, mechanical and water vapor barrier properties of sodium caseinate films containing antimicrobials and their inhibitory action on *Listeria monocytogenes*. *Food Hydrocolloids*, **22**(3): 373–386.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

31. MIN, S., J.M. KROCHTA, and T.R. RUMSEY (2007), Diffusion of thiocyanate and hypothiocyanate in whey protein films incorporating the lactoperoxidase system. *J. Food Eng.*, **80**(4): 1116–1124.
32. SOUTHWARD, C.R. and N.J. WALKER (1980), The manufacture and industrial use of casein. *NZ J. Dairy Sci. Technol.*, **15**: 201–217.
33. COLLINS, J.H. (1952), *Casein Plastics and Allied Materials*, London: The Plastics Institute.
34. MUNRO, P.A., C.R. SOUTHWARD, and P.D. ELSTON (1980), The effect of casein manufacturing variables on the properties of rennet casein plastics. *NZ J. Dairy Sci. Technol.*, **15**: 177–190.
35. PINNER, S.H. (1946), Protein plastics and fibers. A review. *Brit. Plastics*, **18**: 313–318, 353–361.
36. SALZBERG, H.K. (1977), *Handbook of Adhesives*, ed. I. Skiest, New York: Van Nostrand Reinhold, pp. 158–171.
37. BROWNE, F.L. and D. BROUSE (1939), in *Casein and its Industrial Applications*, ed. E. Sutermeister and F.L. Browne, New York: Reinhold, pp. 233–292.
38. DE GRAAF, L.A. and P. KOLSTER (1998), Industrial proteins as a green alternative for 'petro' polymers: Potentials and limitations. *Macromol. Symp.*, **127**: 51–58.
39. SALZBERG, H.K. (1964), Casein. *Encycl. Polym. Sci. Technol.*, **2**: 859–871.
40. PARKER, J. (1930), UK patent.
41. PILZ, E. and O. REGENHARDT (1967), German Democratic Republic, patent application 62133.
42. AXELSSON, A., *et al.* (1991), Performance of batch and continuous reactors with coimmobilized yeast and $\beta$-galactosidase. *J. Chem. Technol. Biotechnol.*, **52**: 227–241.
43. COTTE, J. (1991), Le lait, une matière d'avenir pour la cosmétique. *Lait*, **71**: 213–224.
44. SALZBERG, H.K. (1967), Processed milk casein for hair and skin cosmetics. *American Perfumer and Cosmetics*, **82**: 41–50.
45. SABATO, S.F., N. NAKAMURAKARE, and P.J.A. SOBRAL (2007), Mechanical and thermal properties of irradiated films based on tilapia proteins. *Radiat. Phys. Chem.*, **76**: 1862–1865.
46. SCHOLZ, H.A. (1953), History of water-thinned paints. *Ind. Engng Chem.*, **45**: 710–711.
47. WARTH, A.H. and F.L. BROWNE (1939), in *Casein and its Industrial Applications*, ed. E. Sutermeister and F.L. Browne, New York: Reinhold, pp. 315–353.
48. FLINN, D.P. (1865), US patent 50068.
49. SALZBERG, H.K. (1954), Casein stabilizers for latex paints. *Paint Varn. Product.*, **44**(10): 35–37, 77.
50. GENIN, G. (1958), Casein as a stabilizer for latex paint. *Lait*, **38**: 276–283.
51. RITSON, D.D., D.S. GREIF, and M.E. STONEBRAKER (1963), *Paper Coating Additives*, Tappi Monograph Series No. 22, ed. H.R. Hall, New York: Tappi Press, p. 99.
52. JACKSON, D.L.C. and A.R.A. BACKWELL (1955), Application of protein to wool to reduce felting. I. Casein. *Aust. J. Appl. Sci.*, **6**: 244–254.
53. RYBICKI, E., B. STEELE, and J. HANKIEWICZ (1999), Modified casein as a protective colloid in the washing bath. *Fibres Text. East. Eur.*, **7**(2): 56–60.
54. ZAWADZKI, A. (1999), Antistatic finishing of synthetic fiber textiles. Polish patent 176001B.
55. FRONTCZAK-WASIAK, I. (1999), Evaluation of the usefulness of modified casein as


IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

sizing agent for warp threads. *Fibres Text. East. Eur.*, **7**(2): 53–55.
56. GENIN, G. (1937), The fabrication of artificial wool. *Lait*, **17**: 949–955.
57. COURTAULDS, L.T.D. and R.L. WORMELL (1944), UK patent 564591.
58. KOCH, P.A. (1954), Casein fibers: Fibrolane, Merinova, Caslen. *Fibres Nat. Synthet.*, **15**: 242–244, 248.
59. PETERSON, R.F., R.L. MCDOWELL, and S.R. HOOVER (1948), Continuous-filament casein yarn. *Text. Res. J.*, **18**: p. 744–748.
60. COUPLAND, J.N., *et al.* (2000), Modeling the effect of glycerol on the moisture sorption behavior of whey protein edible films. *J. Food Eng.*, **43**: 25–30.
61. MCHUGH, T.H., J.F. AUJARD, and J.M. KROCHTA (1994), Plasticized whey protein edible films: Water vapor permeability properties. *J. Food Sci.*, **59**(2): 416–423.
62. MCHUGH, T.H. and J.M. KROCHTA (1994), Milk-protein-based edible films and coatings. *Food Technol.*, **48**(1): 97–103.
63. SHARMA, S., J.N. HODGES, and I. LUZINOV (2008), Biodegradable plastics from animal protein coproducts: Feathermal. *J. Appl. Polym. Sci.*, **110**: 459–457.
64. HAN, J.H. and J.M. KROCHTA (1999), Wetting properties and water vapor permeability of whey-protein-coated paper. *Trans. ASAE*, **42**(5): 1375–1382.
65. KOHLER, S. (1991), Zur Verwendung von Milch im Non-food-Bereich. *Lebensmittelindustrie und Milchwirtschaft*, **23**: 696–702.
66. DALEV, P.G. (1994), Utilization of waste whey as a protein source for production of iron proteinate: an antianemic preparation. *Bioresource Technol.*, **48**: 75–77.
67. AIDER, M. and D. DE HALLEUX (2007), Isomerization of lactose and lactulose production: Review. *Trends Food Sci. Technol.*, **18**(7): 356–364.
68. GONZALEZ SISO, M.I. (1996), The biotechnologycal utilization of cheese whey: a review. *Bioresource Technol.*, **57**(1): 1–11.
69. HOBMAN, P.G. (1984), Review of processes and products for utilization of lactose in deproteinated milk serum. *J. Dairy Sci.*, **67**: 2630–2653.
70. THELWALL, L.A.W. (1985), Developments in the chemistry and the chemical modification of lactose, in *Developments in Dairy Chemistry – 3. Lactose and Minor Constituents*, ed. P.F. Fox, London: Elsevier Applied Science, p. 35.
71. ZADOW, J.G. (1984), Lactose: properties and uses. *J. Dairy Sci.*, **67**: 2654–2679.
72. DEVAUX, W. (1993), Whey-permeates and lactose derivatives – future utilization in non-food applications. *International Food Ingredients* (1–2): 39–44.
73. TIMMERMANS, E. (1996), *Lactose: its Manufacture and Physico-chemical Properties*, Carbohydrates as Organic Raw Materials III, developed from a Workshop held in Wageningen, The Netherlands, 28–29 November 1994, pp. 93–113.
74. MENDEZ, A. and A. OLANO (1979), Lactulose: a review of some chemical properties and applications in infant nutrition and medicine. *Dairy Sci. Abstr.*, **41**: 531–535.
75. HARJU, M. (1993), Production and properties of lactulose, lactitol and lactobionic acid. *Bull. Int. Dairy Fed.*, **289**: 27. International Dairy Federation, Brussels.
76. SAIJONMAA, T., *et al.* (1978), Preparation of milk sugar alcohol, lactitol. *Milchwissenschaft*, **33**: 733–736.
77. RUDOLFOVA, J. and L. CURDA (2005), Galactooligosaccharides as prebiotics and their production from lactose. *Chemicke Listy*, **99**(3): 168–174.
78. YANG, S.T. and E.M. SILVA (1995), Novel products and new technologies for use of a familiar carbohydrate, milk lactose. *J. Dairy Sci.*, **78**: 2541–2562.
79. YANG, S.T., H. ZHU, and E.M. SILVA (1993), *Production of value-added products from agricultural and food processing byproducts*, in *Biotechnology in the 21st century*, ed. C. Ayyanna, New Delhi: Tata McGraw-Hill, pp. 47–68.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

80. VISWANATHAN, T., D. BURRINGTON, and T. RICHARDSON (1984), Preparation of rigid, low density, flame retardant polyurethane foams from whey permeate. *J. Chem. Technol. Biotechnol.*, **34B**: 52–56.
81. VISWANATHAN, T. and A. TOLAND (1991), Whey modified phenol-formaldehyde resins as plywood adhesives. *Carbohyd. Polym.*, **15**: 41–49.
82. KILARA, A. and M.T. PATEL (1992), Whey and lactose fermentation, in *Whey and Lactose Processing*, ed. J.G. Zadow, London: Elsevier Applied Science, pp. 409–448.
83. MORR, C.V. (1992), Whey utilization, in *Whey and Lactose Processing*, ed. J.G. Zadow, London: Elsevier Applied Science, p. 133.
84. DEMIREL, B., O. YENIGUN, and T.T. ONAY (2005), Anaerobic treatment of dairy wastewaters: a review. *Process Biochemistry* (Oxford), **40**(8): 2583–2595.
85. KOSARIC, N. and Y.J. ASHER (1985), The utilisation of cheese whey and its components. *Adv. Biochem. Engng*, **35**: 25–60.
86. MAWSON, A.J. (1994), Bioconversions for whey utilization and waste abatement. *Bioresource Technol.*, **47**(3): 195–203.
87. ATKINSON, B. and F. MAVITUNA (1983), *Biochemical Engineering and Biotechnology Handbook*, London: Macmillan, pp. 890–931.
88. GOBLOS, S., *et al.* (2008), Comparison of the effectivities of two-phase and single-phase anaerobic sequencing batch reactors during dairy wastewater treatment. *Renewable Energy*, **33**(5): 960–965.
89. GELEGENIS, J., *et al.* (2007), Optimization of biogas production by co-digesting whey with diluted poultry manure. *Renewable Energy*, **32**(13): 2147–2160.
90. OZMIHCI, S. and F. KARGI (2008), Ethanol production from cheese whey powder solution in a packed column bioreactor at different hydraulic residence times. *Biochem. Eng. J.*, **42**(2): 180–185.
91. BARRY, J.A. (1982), Alcohol production from cheese whey. *Dairy Ind. Int.*, **47**(10): 19–22.
92. SILVEIRA, W.B., *et al.* (2005), Ethanol production from cheese whey permeate by Kluyveromyces marxianus UFV-3: A flux analysis of oxido-reductive metabolism as a function of lactose concentration and oxygen levels. *Enzyme Microb. Technol.*, **36**(7): 930–936.
93. STRONACH, S.M., T.M. RUDD, and J.N. LESTER (1986), *Anaerobic Digestion Process in Industrial Wastewater Treatment*, Berlin: Springer-Verlag.
94. HWANG, S. and C.L. HANSEN (1998), Characterization of and bioproduction of short chain organic acids from mixed dairy-procesing wastewater. *Trans. ASAE*, **41**(3): 795–802.
95. VADLANI, P.V., A.P. MATHEWS, and G.S. KARR (2008), A two-stage fermentation process: production of propionate and acetate salt as road deicer from cheese whey. *Biological Engineering*, **1**(1): 95–104.
96. MORALES, J., J. CHOI, and D. KIM (2006), Production rate of propionic acid in fermentation of cheese whey with enzyme inhibitors. *Environ. Prog.*, **25**(3): 228–234.
97. TANGO, M.S.A. and A.E. GHALY (1999), Amelioration of lactic acid production from cheese whey using micro-aeration. *Biomass Bioenergy*, **17**: 221–238.
98. GONZALEZ, M.I., *et al.* (2008), Lactic acid recovery from whey ultrafiltrate fermentation broths and artificial solutions by nanofiltration. *Desalination*, **228**(1–3): 84–89.
99. DATTA, R. and S.P. TSAI (1997), Lactic acid production and potential uses: a technology and economics assessment. *ACS Symp. Ser. (Fuels and Chemicals from Biomass)*, **666**: 224–236.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

100. MIYAMOTO, Y., T. OOI, and S. KINOSHITA (2000), Production of lactobionic acid from whey by *Pseudomonas* sp. LS13-1. *Biotechnol. Lett.*, **22**(5): 427–430.
101. LOPEZ RIOS, C.A., *et al.* (2006), Production of citric acid with *Aspergillus niger* NRRL 2270 from milk whey. *Dyna* (Medellin, Colombia), **150**: 39–57.
102. MUKHOPADHYAY, R., *et al.* (2005), Production of gluconic acid from whey by free and immobilized *Aspergillus niger*. *Int. Dairy J.*, **15**(3): 299–303.
103. SCHWARTZ, R.D. (1993), Biopolymers from whey. *Bull. Int. Dairy Fed.*, **212**: 56. International Dairy Federation, Brussels.
104. PAPOUTSOPOULOU, S.V., L.V. EKATERINIADOU, and D.A. KYRIAKIDIS (1994), Genetic construction of *Xanthomonas campestris* and xanthan gum production from whey. *Biotechnol. Lett.*, **16**: 1235–1240.
105. THORNE, L., L. TANSEY, and T.J. POLLOCK (1988), Direct utilization of lactose in clarified cheese whey for xanthan gum synthesis by *Xanthomonas campestris*. *J. Ind. Microbiol.*, **3**(3): 321–328.
106. FRENGOVA, G.I., *et al.* (2002), Exopolysaccharides produced by lactic acid bacteria of kefir grain. *Z. Naturforsch.*, **57**: 805–810.
107. BERGMAIER, D., C.P. CHAMPAGNE, and C. LACROIX (2003), Exopolysaccharide production during batch cultures with free and immobilized *Lactobacillus rhamnosus* RW-9595M. *J. Appl. Microbiol.*, **95**(5): 1049–1057.
108. ROSALAM, S. and R. ENGLAND (2006), Review of xanthan gum production from unmodified starches by *Xanthomonas compestris* sp. *Enzyme Microb. Technol.*, **39**: 197–207.
109. DAVIES, J. (1984), Oil from whey. *Food Technol. N.Z.*, **38**(10): 33.
110. KAYLEGIAN, K.E. (1995), Functional characteristics and nontraditional applications of milk lipid components in food and nonfood systems. *J. Dairy Sci.*, **78**(11): 2524–2540.
111. AMERCHOL CORPORATION (1994), Cremerol HMG product brochure, Edison, NJ.
112. GREENER DONHOWE, I. and O. FENNEMA (1994), Edible films and coatings: characteristics, formation, definitions, and testing methods, in *Edible Coatings and Films to Improve Food Quality*, ed. J.M. Krochta, E.A. Baldwin and M.O. Nisperos-Carriedo, Lancaster, PA: Technomic Publishing, p. 1.
113. SHELLHAMMER, T.H., T.R. RUMSEY, and J.M. KROCHTA (1997), Viscoelastic properties of edible lipids. *J. Food Eng.*, **33**: 305–320.

IP Address: 129.132.208.100

© Woodhead Publishing Limited, 2010

# Index


A2 hypothesis, 84, 85
$\alpha$-lactalbumin, 77–8
$\alpha$-lactalbumin, 332, 454, 456, 459, 461, 481
A2 milk, 84–5
$\alpha$-tocopherol, 235
acetaldehyde, 419, 422
acid extraction, 210–11
adenosine monophosphate, 468
ADSA *see* American Dairy Science Association
ADSA score cards, 160
aflatoxin B1, 378
aflatoxin M1, 378
aflatoxins, 378
  absorption, 378–9
  limit, 378
African Buffalo *see Syncerus*
AgResearch, 83, 87
air quality, 356–7
air temperature, 356–7
Ajinomoto, 329
albumin, 406
alkyl phenol, 381
all-plastic containers, 139
all-rac-$\alpha$-tocopheryl acetate, 120
all-trans $\beta$-carotene, 231
Alpine, 318, 332, 334, 337
alternative feeds, 369–70
aluminium, 153
aluminium cans, 138
American Dairy Science Association, 160
amino acids, 459
ammonia, 459
aneurin *see* thiamine
Anglo-Nubian, 318
anion exchange chromatography, 213
AOP, 442–3
apocrine secretory process, 306
aqueous phase, 211
Aralac, 481
ascorbate, 102
ascorbic acid, 102, 245
ashing, 210
Asian Buffalo *see Bubalus bubalis*
*Aspergillus*, 378
*Aspergillus glavus*, 378
*Aspergillus parasiticus*, 378
*Aspergillus* spp, 423
Assaf ewes, 369
atomic absorption, 212
atomic emission, 212
auto-oxidation, 106
Awassi ewes, 349–50, 365
Ayrshire, 298

$\beta$-carotene, 81
$\beta$-casein, 354, 426, 443
$\beta$-galactosidase, 462
$\beta$-lactoglobulin
  designer milk component, 76–7
  effect of heat treatment, 422, 424, 444
  goat milk production improvement, 332
  goat milk yoghurt texture enhancement, 328
  high pressure, 466
  infant food and allergenicity, 463, 464, 465, 468
  milk quality requirements for cheesemaking, 434, 441
  non-food uses, 481


© Woodhead Publishing Limited, 2010

*Bacillus*, 99
*Bacillus cereus*, 102–3, 105
  pasteurised and ESL milk spoilage, 99–100
  sources of spores in raw and pasteurised milk, 103
*Bacillus sporothermodurans*, 108, 115
  heat inactivation and denaturation curves in UHT region, 119
*Bacillus subtilis*, 100
bacterial lipases, 322
bacterial mastitis, 426
Bactocatch process, 444
baker's yeast, 484
bifidobacteria, 329, 462
*Bifidobacterium infantis*, 418, 424
*Bifidobacterium* spp, 424, 482
bifidogenic effect, 457
bioactive peptides, 78–9
biodiversity, 263
biofilm, 104, 122
biofuels, 300
biogas, 484
biotin, 242
biotin deficiency, 242
boilers, 264–6, 272–3
Brown Swiss, 298, 319
*Brucella*, 352
*Bubalus bubalis*, 403
buck effect, 305
bucket-milking machines, 375
buffalo milk
  average fat content, 412–13
  buffalo distribution and milk production among major buffalo farming countries, 403
  changes in chemical composition of commingled water buffalo milk, 411
  chemical composition, 404–6
  considerations to be taken in product processing, 406–7
  dairy management and milk production, 408–9
  factors that influence the yield and composition, 410–12
  factors to consider for milk production and reproductive capacity of buffalo, 412–14
  feeding management, 409–10
  generic variants of caseins and whey proteins, 436
  gross composition and pH, 433
  improvement, 402–14
  milk products, 406–7
  protein content, 413
  vs bovine milk physiochemical properties, 405
  vs cow's milk gross composition, 410
buffer feeding, 294
butter, 406
butter oil, 406
buttercups, 427
butterfat, 404
buttermilk powder, 328
butyric acid, 438

cadmium, 379
calcium, 11–12, 80, 299, 324, 404, 406, 427
  addition in milk, 215–17
  and milk products for bone health and osteoporosis prevention, 35–6
  and osteoporosis, 29–30
  bioavailability
    food and calcium salts, 33
    milk and milk products, 32–5
  chelatants addition in milk, 219
  concentrations in foods, 32
  effect on body weight and fat, 46
  fortified milk products, 37
  homeostasis regulation, 30–2
  mineral quantification
    catalase enzyme electrode, 213
    selective electrodes, 213
    spectrophotometric and fluorimetric methods, 213
    titration, 212–13
  protection from obesity, 44–7
    evidence for an inverse relation between intake and obesity, 44–6
    mechanisms of anti-obesity effect, 46–7
    relevance of anti-obesity effect, 47
  quantification by catalase enzyme electrode, 213
  recommended intakes, 31
  requirements and bioavailability, 30
calcium chloride, 349
calcium phosphate, 443
calcium soap of olive oil, 365
calcium soap of palm oil, 365
calcium soaps of fatty acids, 365
California Mastitis Test, 306
camel milk, 425, 459
Camembert cheese, 325
cancer, 17–18
capillary electrophoresis, 214
caprine milk, 426
carbon dioxide, 107
  cheese milk treatment, 445
carbon dioxide emissions, 261
Carbowax, 194
cardiovascular disease
  and hypertension, 37–44
  definition, 38
  other factors in milk and milk products with positive impact on risk, 40–3
    inflammation, 42–3
    insulin sensitivity, 41–2
    lipids, 40–1
    obesity, 40

© Woodhead Publishing Limited, 2010

carotene, 102, 406
carotenoids, 14
carrageenan, 327
casein micelles, 407, 421, 425, 435, 443, 445
  structure, 110
casein-whey ratio, 454, 455, 459
caseinates, 404, 481
caseinophosphopeptides, 34
caseins, 34
  bitterness reduction, 468, 477
  buffalo milk, 404, 406
  goat milk production improvement, 334
  goat milk yoghurt texture enhancement, 328
  infant food and allergenicity, 463
  other non-food applications, 481, 483
  technical applications, 477
casein:whey protein ratio, 421, 424
catalyse enzyme electrode, 213
CCP *see* colloidal calcium phosphate
*Ceratonia siliqua*, 369
Cheddar cheese, 289, 326, 327, 407, 437, 438
cheese whey, 482–3
cheesemaking, 334
  effects of milk on yield and quality, 435, 437–42
    animal diet, 440–1
    genetic variants, 441–2
    lactation, 439–40
    seasonal variation, 442
    somatic cell count, 439
  fatty acid composition of different cheese milks, 437
  influence of milk preparation, 442–6
    CCP and milk serum phosphates balance, 443
    cheese milk treatment with carbon dioxide, 445
    cold storage, 442–3
    heat treatment, 443–4
    high pressure treatment of cheese milk, 445
    membrane filtration, 444–5
    microfiltration, 445–6
  milk microbial quality, 435, 437–9
    detrimental bacteria, 438–9
    lactic acid bacteria, 437
    pathogenic bacteria, 437–8
  milk quality requirements, 433–46
  ranges of milks used, 433–4
chemical deterioration
  future trends, 122
  pasteurised and ESL milk, 101–2
    copper-induced oxidation, 101
    light-induced, 101
    prevention in milk, 97–122
    vitamin-linked, 102
  prevention in milk, 97–122
  UHT and sterilised milk, 114–15
Chèvre cheese, 325
chicory extract, 462
chloride, 213–14
  quantification by spectrophotometric method and titration, 214
cholecalciferol, 233
choline, 459
*Chrysanthemum coronarium*, 372
*Cicer arietinum*, 369
*cis*-9, *trans*-11-octadecadienoic acid, 337
citrate, quantification by
  ionic chromatography and capillary electrophoresis, 213–14
  spectrophotometric methods, 214
*Citrus limon* L., 370
CLA *see* conjugated linoleic acid
clean-in-place systems, 272
clean silage, 438
cleaning-in-place systems, 422
*Clostridium difficile*, 83
*Clostridium* spp, 374
*Clostridium tyrobutyricum*, 438
clover, 290
clover swards, 287
CMA *see* cow's milk allergy
coagulase-negative staphylococcus, 376
coagulating agents, 433
cobalamin deficiency, 245
cobalamins, 244
cocksfoot, 289–90, 293
Codex, 307
coefficient of performance, 267
cold microfiltration, 446
cold storage, 442–3
Coldsoft, 267
colloidal calcium phosphate, 425, 435, 444
  and milk serum phosphates balance, 443
colostrum, 82–3, 439
  levels of immunoglobulins and growth factors, 77
Columbia ewe, 353
column chromatography, 468
Comisana, 351
compactors, 275
complement fixation, 458
complex glycosylation, 461
composting, 275
Compusense five software, 166
Comté cheese, 441
condensed tannins, 369
conjoint analysis, 172
conjugated linoleic acid, 10, 40, 79, 86–7, 291, 349, 360, 406
  content variability, 360
  in improving goat milk, 337–8
  protection against obesity, 47–8
cooling towers, 272–3
copper-complex method, 214
copper toxicity, 299

© Woodhead Publishing Limited, 2010

Corriedale ewe, 353
corrinoids, 244
Cottage cheese, 433
cow milk
  generic variants of caseins and whey proteins, 436
  gross composition and pH, 433
  hydrophilic vitamins, 237–46
    group B vitamins, 237–45
    vitamin C, 245–6
  lipophilic vitamins, 231–7
    vitamin A, 231–3
    vitamin D, 233–5
    vitamin E, 235–6
    vitamin K, 236–7
  naturally occurring vitamins, 231–46
  salts partition, 208
  vs buffalo milk
    gross composition, 410
    physiochemical properties, 405
cow's milk allergy, 463
crates, 144
cream, 406
creatine, 459
creatinine, 459
crimson clover, 287, 288
Crohn's disease, 438
crude olive cake, 365
crude protein, 367
CSFA *see* calcium soaps of fatty acids
CSIRO, 361
*CSN1S1*, 334
*CSN1S2*, 334
CSPO *see* calcium soap of palm oil
cyanocobalamin, 244–5

*D*-value, 119
Dairy Farmers, 275
dairy farming, 253–63
  air emissions and energy consumption, 259–62
  changing soil characteristics, 253–7
  farm wastes and chemicals, 262
  habitat and biodiversity loss, 263
  water contamination and consumption, 258–9
dairy fat, 42
Dairy HACCP Safety System, 307
dairy industry
  dairy farming environmental impacts, 253–63
    air emissions and energy consumption, 259–62
    changing soil characteristics, 253–7
    direct financial savings from soil conservation work, 255
    farm wastes and chemicals, 262
    financial savings from rotational grazing, 256
    habitat and biodiversity loss, 263
    water contamination and consumption, 258–9
  dairy processing environmental impacts, 263–76
    compressed air leaks cost, 269
    cost of water loss from leaking equipment, 271
    fuel savings from installing online oxygen control, 265
    heat losses from steam lines, 266
    methane and energy yields from biogas digestion, 269
    non-renewable fuel and greenhouse gas emissions consumption, 263–70
    solid waste, 273–6
    soot and scale effects on heat transfer, 265
    waste minimisation triangle, 274
    water use, 270–3
  effluent dollar value
    from cows fed maize on feed pad, 258
    from cows on all grass system, 258
  environmental challenges, 252–3
  managing environmental impact, 252–76
Dairy Oh!, 86
dairy processing
  environmental impacts, 263–76
    solid waste, 273–6
    water use, 270–3
  non-renewable fuel and greenhouse gas emissions consumption
    boilers, 264–6
    compressed air, 268–9
    energy management, 264
    evaporators and driers, 264
    other opportunities, 269–70
    refrigeration, 266–8
dairy products
  chemical and physical hazards, 311
  microbiological hazards, 311
Dairybal, 256
Danish organic herds, 300
DASH diet, 17
Delle Lange, 351
delta-9-desaturase enzyme, 360
demineralisation, 221
descriptive analysis, 163
designer milks
  functional components, 74–81
    lipids, 79–80
    minerals, 80–1
    oligosaccharides, 80
    other small molecules, 81
    protein and peptide components, 75–9
  functional foods from milk, 74–89
  future trends, 88–9
  production and application, 81–8
    A2 milk, 84–5
    colostrum, 82–3
    hyperimmune milk, 83

© Woodhead Publishing Limited, 2010

milk protein hydrolysates, 85
milk with high melatonin levels, 87–8
milk with unmodified fat composition, 85–7
selenium-enriched milk, 87
diabetes mellitus type 2, 16
diafiltration, 220, 446
dialdehydes, 479
*Diet, Nutrition and the Prevention of Chronic Diseases*, 5
diisocyanates, 479
dimethyl-diphenylpolysiloxane, 194
dioxins, 377, 380
direct solvent extraction, 191–2
discrimination tests, 162–3
DOC/DOP products, 381
driers, 264
drums, 144
Dutch milk, 442

East Friesian sheep, 356
EEC Council Directive 82/711/EC, 150
EEC Council Directive 90/128/EEC, 150
Effective Use of Water on Dairy Farms, 259
egg white, 328
electroheating, 107
electronic noses, 196–7
endocrine disrupting compounds, 380
energy balance, 359, 363
energy management, 264
ensiled crops, 290
enzymatic hydrolysis, 464–5
epitopes, 465
ergocalciferol, 233
*Escherichia coli*, 437, 467
*Escherichia coli* O157, 423
*Escherichia coli* O157:H7, 437
ESL milk *see* extended shelf-life milk
ethanolamine, 459
ethylene vinyl alcohol, 480
EU directive 1662/2006, 99
EU Directive 1994/62/EC, 152
EU Directive 2002/72/EC, 150
EU Directive 2004/12/EC, 152
EU Synoptic Document No. 7, 150
evaporation, 330
under vacuum, 420
evaporators, 264
Every Drop Counts programme, 270
ewe milk
generic variants of caseins and whey proteins, 436
gross composition and pH, 433
exopolysaccharides, 327, 483, 484
extended shelf-life milk
pasteurisation, 98
spoilage, 99–107

factor loadings, 173
factor scores, 173
fat, 349
fat-soluble vitamins, 14–15
fatty acids, 349
effects on body weight in overweight subjects, 48
feedback inhibitor of lactation, 354
feta cheese, 325
fibreboard cans, 142
fibreboard composites, 142
fibreboard-corrugated board, 142–3
Fibrolane, 481
films, 143–4
fish meals, 368
fish oil, 338
flame ionisation detector, 194
flash process, 323
flavomycin, 373
flavour scalping, 151
fluorimetric methods, 213
fodder beet, 289
folates, 242–4
folic acid, 242–3
Food and Agriculture Organisation, 412
Food and Drug Administration, 306, 313
food spoilage, 98
forage maize, 287, 289
forage peas, 288
formaldehyde, 479, 481
form–fill–seal system, 428–9
free fatty acid, 112–13, 372
phase, 194
solubility in water, 113
fresh acid French-type cheese, 325
fructo-oligosaccharides, 462
fumonisin B, 378
functional food
definition, 75
fusariotoxins, 378
*Fusarium*, 378

$\gamma$-irradiation, 479
galacto-oligosaccharides, 462, 482
Galalith, 480
Garganica, 334
gas chromatography-mass spectrometry, 193–5
gas chromatography-olfactometry, 195
GC-MS *see* gas chromatography-mass spectrometry
G2cNAc1 *see* N-acetyl-$\delta$-glucosamine
GCO *see* gas chromatography-olfactometry
gelation, 109
Gemir, 407
Georgia Small Ruminant Research and Extension Centre, 323–4
ghee, 406
glass, 153
glass bottles, 138–9
Global Prevalence of Vitamin A Deficiency, 233

© Woodhead Publishing Limited, 2010

globulin, 406
glutamine, 329
glutaraldehyde, 479
glyceraldehyde, 479
glycerol, 478
glycosylation, 460
goat colostrum, 320
goat milk
  allelic frequencies at CSNS1 locus in Hungarian, Saanen, Alpine and local Spanish breeds, 333
  basic nutrient contents of commercial US products, 331
  changes in milk yield, fat and protein contents, 318
  chemical, bacteriological and temperature standards, 308
  developments in processing techniques, 323–32
    cosmetic goat milk products, 330, 332
    evaporated and powdered goat milk products, 330
    freezing goat milk curds and cheeses, 325
    frozen goat milk products, 330
    heat stability in relation to processing techniques, 324
    nutritional fortification, 326
    pasteurisation and heat treatment methods, 323–4
    reduced fat goat milk products, 326–7
    ultrafiltration, 324–5
    yoghurt processing, 327–9
  factors affecting composition and yield of milk, 316–21
    breed, 317–18
    colostrum, 320
    diet, 317
    disease, 320
    environmental temperature, 319–20
    lactation stage, 318–19
    other factors, 320–1
    season, 319
  factors affecting the quality before and after milking, 321–3
    after milking, 322–3
    before and during milking, 321–2
  five-point mastitis control program, 322
  generic variants of caseins and whey proteins, 436
  gross composition and pH, 433
  guidelines for contamination prevention, 313–16
    pasteurisation, 314–15
    post-pasteurisation contamination, 315–16
    vat pasteurisation, 315
  improvement, 304–38
  improving production, 332–8
    effect of milk protein polymorphism on renneting properties, 334–5
    increasing CLA and other nutrient contents, 337–8
    protein polymorphisms, 332–4
    somatic cell principles, 336–7
  keeping and improving flavour, 335–6
    general background of goat milk flavour, 335
    prevention of off-flavour sources, 335–6
  key issues in improvement, 304–5
  main contamination sources, 316
  quality control guidelines for microbiological standards in dairy foods, 309
  quality goat milk production, 305–16
    essential control systems, 307–10
    general principles, 305–6
    HACCP plans and hazard components, 310–13
    regulatory standards, 306–7
  sources of off-flavours, 336
  types of market, 305
goat milk hand lotion, 330
goat milk ice, 330
goat milk soap, 330
goaty flavour, 306, 307, 335
goaty odour, 335
good animal husbandry practices, 414
Good Hygiene Practices, 106
Good Manufacturing Practices, 106
gossypol, 479
Gouda, 407
gout, 18
Grade A Pasteurised Milk Ordinance, 306
grass, 294, 300
grazing system, 293
Greek-style yoghurts, 420
ground nut protein, 328
growth factors, 464
  human milk, 458
  levels in milk and colostrum, 77
  NPN, 459
growth hormone, 361
Guernsey, 298

HACCP *see* Hazard Analysis Critical Control Point
HAMLET *see* human $\alpha$-lactalbumin made lethal to tumour cells
Hampshire ewe, 353
Hazard Analysis Critical Control Point, 106
  commercial fluid milk processing procedures flowchart, 314
  milking and cheese manufacture processes flow diagram, 313
  plans and hazard components, 310–13
  prerequisite areas of developing an HACCP plan, 310

© Woodhead Publishing Limited, 2010

processing flow diagram, 312
seven principles, 310
HDPE *see* high density polyethylene
headspace-solid phase microextraction, 191
Health Professionals Follow-up Study, 7
heat treatment, 443–4, 461
heavy metals, 377, 379
hedonic scale, 9-point, 163, 164
*Hedysarum coronarium*, 369, 372
HHRS *see* highly heat resistant spore former
high density polyethylene, 139–40
high impact polystyrene, 140–1
high-pressure treatment, 462
High Temperature–Short Time system, 98, 314, 323, 422
high vacuum distillation, 192
highly heat resistant spore former, 108, 115–16
Hilton Hotel Caraca, 407
HIPS *see* high impact polystyrene
Holstein breed, 298, 319
Holstein–Friesian cows, 299
Honeywell Farms, 268
Honolulu Heart Program Study, 7
HTST *see* High Temperature–Short Time system
human $\alpha$-lactalbumin made lethal to tumour cells, 77
human colostrum, 458
human milk, 455–9
growth factors, 458
immunoglobulins, 458
MFGMP, 456
NPN, 459
nucleotides, 456–7
oligosaccharides, 457–8
prebiotic effect, 457
proteome, 456
sleep-inducing effect, 457
Hungarian milking goats, 334
hybrid ryegrass, 288
hydrogen bond, 477
hydrogen peroxide, 106
hyperimmune milk, 83
hypertension, 17
and milk, 39–40
and overall cardiovascular disease, 37–44
hypoallergenic infant formula, 464–5

ice cream, 406
IDF *see* International Dairy Federation
IDFA *see* International Dairy Foods Association
immunoglobulin A, 456, 458
immunoglobulin G, 458
immunoglobulin M, 458
immunoglobulins, 76
human milk, 458, 464
levels in milk and colostrum, 77
in-carton production, 429
incineration, 154–5
indole, 369
induced lipolysis, 322
infant food
and allergenicity, 463–6
high pressure, 466
IgE response during allergy, 464
technologies to produce hypoallergenic infant formula, 464–5
G2cNAc1 profile of freshly isolated cheese-whey, 461
humanisation, 459–62
casein–whey protein ratio and minor components, 459–60
enhancing function vs mimicking composition, 462
new preserving technologies, innovative steam injection, 461–2
processing and (bio)functionality, 460–1
RP-HPLC protein separation profiles, 460
infant formulas
analytical tools and models, 463
bioactive peptides and their potential effects, 467
dairy perspective of trends, 454–70
human milk, 455–9
humanisation of infant food, 459–62
infant food and allergenicity, 463–6
milk protein hydrolysates benefits, 466–9
inflammation, 42–3
inflammatory bowel diseases, 55–6
Ingman Dairy, 88
innovative steam injection, 120
inorganic phosphate, 213–14
instrumental measurement
colour, 197–8
flavour measurement, 188–97
flavour compound isolation/extraction, 188–91
instrumental methods of analysis, 193–7
other isolation/extraction methods, 193
solvent extraction-distillation methods, 191–3
milk flavour and colour, 181–98
future trends, 198
off-flavours and flavour defects in fluid and dried milks, 186–7
potent odourants identified by GCO
in nonfat and wholefat dried milks, 184–5
in raw, pasteurised and UHT fluid milks, 183
insulin resistance syndrome *see* metabolic syndrome
insulin sensitivity, 41–2
Integrale Kwaliteitszorg Melk – Dairy Quality Assurance Scheme, 116

© Woodhead Publishing Limited, 2010

International Association of Infant Food Manufacturers, 455
International Dairy Federation, 307
International Dairy Foods Association, 307, 313
ion exchange chromatography, 220–1, 459
ionic chromatography, 213
iron, 406
irritable bowel syndrome, 55–6
isoelectrofocusing, 334–5
Italian goat breeds, 334
Italian ryegrass, 288

jacket/in-tank cooling system, 428
Jarlsberg cheese, 445
Jersey, 298–9, 319
Johne's disease, 438
Just About Right scale, 164

κ-casein, 351, 422, 428, 434, 435, 441, 442, 444, 461, 463
kale, 288–9

L-phenylalanine, 378
Labelled Affective Magnitude scale, 164
Lacaune breed, 351, 359
Lacaune breeding programme, 361
lactadherin, 456
lactic acid, 479, 482
lactic acid bacteria, 418, 434, 437
*Lactobacillus acidophilus*, 329, 418, 424, 429
*Lactobacillus casei*, 329
*Lactobacillus delbrueckii* subsp *bulgaricus*, 327, 418, 423, 426, 427
*Lactobacillus* spp, 352
lactococcal extracellular envelope protease, 465
*Lactococcus* spp, 352
lactoferricin, 467
lactoferrin, 76, 459, 461, 463, 464
lactoflavin *see* riboflavin
lactoperoxidase, 76, 426, 459, 461, 464
lactose, 10, 53, 404, 459, 462, 477
  non-food uses, 481–3
lactose intolerance
  evidence for beneficial effects of probiotics and probiotic milk products, 53–4
lactose powder, 273, 275
lactosyl urea, 482
lactosylation, 460
LaMancha, 318
Lannital, 481
lauric acid, 483
LDPE *see* low-density polyethylene
LDPE pouches, 141
legumes, 288, 290
*Leuconostoc*, 352
life cycle, 152
linseed, 368
lipases, 112
lipids, 40–1, 79–80
  conjugated linoleic acids, 79
  milk polar lipids, 80
  ω-3 long-chain polyunsaturated fats, 79
lipolysis, 322
*Listeria*, 322, 352
*Listeria monocytogenes*, 437
lithium bromide absorption chiller, 268
live tractors of the East, 412
LLDPE pouches, 141
logistic regression, 172
*Lolium rigidum*, 372
long-chain fatty acid, 363
longevity, 299
low-density lipoprotein, 9
low-density polyethylene, 143
Low Temperature Holding system, 422
LTLT autoclave treatment, 323–4
Lucerne, 287, 288, 293
*Lupinus albus*, 369
lysine, 329, 368
lysozyme, 464

machine milking, 408
magnesium, 13, 212–13
Maillard reactions, 115, 460
maize silage, 289
Malaguena, 334
Maltese, 334
maltodextrin, 459
MAP *see Mycobacterium paratuberculosis*
mass spectrometric detector, 194
Massese, 351
mastitis, 296, 320, 351
  five-point mastitis control program, 322
meadow fescue, 289–90
*Medicago polymorpha*, 372
medium-chain fatty acids, 49
megaloblastic anaemia, 243
melatonin, 81, 87
membrane filtration, 444–5
menadione, 236–7
Merino ewes, 365
merocrine process, 306
metabolic syndrome, 16, 38
  defined limits, 38
methane, 260, 484
methanogenic process, 482
methionine, 367, 368, 422
methyl-cobalamin, 244–5
MFGMP *see* milk fat globule membrane protein
micellar casein, 328
micellar phase, 208–9, 211
micro-encapsulation, 469
microbial lipolysis, 322
microbial spoilage
  future trends, 122

© Woodhead Publishing Limited, 2010

pasteurised and extended shelf-life milk, 99–107
*Bacillus cereus* spores in raw and pasteurised milk, 103
chemical deterioration, 101–2
current methods to prevent spoilage, 104–7
emerging methods to prevent spoilage, 107
factors influencing risk of spoilage, 102–4
microbiological spoilage, 99–101
prevention in milk, 97–122
UHT and sterilised milk spoilage, 108–21
aprX-lipA operon structure in *Pseudomonas fluorescens* B52, 112
casein micelle structure, 110
chemical deterioration, 114–15
correlation between phospholipolytic and lipolytic activity, 114
current methods to prevent spoilage, 118–20
emerging methods to prevent spoilage deterioration, 120–1
enzymatic reaction of lipase catalysing triacylglycerol hydrolysis, 113
factors influencing spoilage, 115–18
FFA solubility in water, 113
heat inactivation and denaturation curves in UHT region, 119
impact of spoilage enzymes on quality, 109–14
microbial contamination and spoilage, 108–9
psychotrophic bacterial growth in raw milk, 117
vitamin E forms, 121
microbial transglutaminase, 329
*Micrococcus*, 352
microfiltration, 105, 444, 445–6
microorganisms, 146
migration, 149–50
milk
analysing and improving mineral content, 207–21
improving mineral content, 215–21
methods for analysis, 210–15
minerals of milk, 207–10
analysing and improving the vitamins levels, 229–48
naturally occurring vitamins in cow's milk, 231–46
techniques to improve vitamin content, 246–7
aqueous phase theoretical concentrations of ions and salts, 208
bone and teeth health, 29–37
bone health and osteoporosis prevention, 35–6
calcium and osteoporosis, 29–30
calcium bioavailability, 32–5
calcium concentrations in foods, 32
calcium-fortified milk products, 37
calcium homeostasis regulation, 30–2
calcium requirements and bioavailability, 30
course of bone mineral density, 35
improving bone- and teeth-protective properties, 36–7
milk products and teeth health, 36
recommended calcium intakes, 31
trabecular structure of osteoporotic tibia, 30
buffalo milk improvement, 402–14
chemical composition, 404–6
dairy management and milk production, 408–9
factors that influence the yield and composition, 410–12
factors to consider for milk production and reproductive capacity of buffalo, 412–14
feeding management, 409–10
milk products, 406–7
cardiovascular disease and hypertension, 37–44
defining metabolic syndrome, 38
general aspects, 37–8
milk and hypertension, 39–40
other factors with positive impact on risk, 40–3
products protecting from risk, 43–4
commercial fluid milk processing procedure, 314
effect of air temperature and quality, 356–7
effects of natural and added milk constituents on gut health, 50–60
beneficial effects, 51
effect of lactobacilli and bifidobacteria on diarrhoea, 55
fermented products, probiotics and gastrointestinal complaints and diseases, 52–6
gut, intestinal microbiota and immunity, 50–1
gut protective constituents, 51
improving milk products for gut health, 58–9
non-yoghurt probiotic food examples, 60
prebiotics, 56–7
probiotics health effects, 52
proof of lack of pro-, pre- or symbiotic milk products, 59–60
safety of pro- and prebiotics, 57–8
effects of packaging on quality and safety, 136–55
environmental issues regarding packaging materials, 151–5

© Woodhead Publishing Limited, 2010

factors affecting milk shelf-life and safety, 145–9
migration and flavour scalping, 149–51
types of packaging materials and their applications, 137–45
factors affecting composition and yield of milk, 317
forms and sources in disparate cultures, 4–5
functional components, 74–81
bioactive peptides, 78
immunoglobulin levels and growth factors, 77
lipids, 79–80
minerals, 80–1
oligosaccharides, 80
other small molecules, 81
protein and peptide components, 75–9
functional foods, 74–89
future trends, 88–9
goat milk improvement, 304–38
developments in processing techniques, 323–32
factors affecting quality, 316–23
improving production, 332–8
key issues in improving goat milk, 304–5
quality goat milk production, 305–16
gross composition and pH of milk from cow, buffalo, goat and ewe, 433
health aspects, 28–60
infant formula trends, 454–70
human milk, 455–9
humanisation of infant food, 459–62
infant food and allergenicity, 463–6
other beneficial properties of milk protein hydrolysates, 466–9
towards optimised composition, 463
known generic variants of caseins and whey proteins, 436
levels of immunoglobulins and growth factors, 77
low-dairy diet disadvantages, 15–19
cancer, 17–18
hypertension/stroke, 17
obesity, 15–16
other possible risks, 18–19
type 2 diabetes/metabolic syndrome, 16
macronutrients, 6–11
carbohydrate, 10–11
fat, 7–10
protein, 6–7
main contamination sources, 316
manipulated functional properties, 81–8
A2 milk, 84–5
colostrum, 82–3
hyperimmune milk, 83
protein hydrolysates, 85
selenium-enriched, 87
with high melatonin levels, 87–8
with modified fat composition, 85–7
minerals, 11–14
calcium, 11–12
potassium, magnesium, phosphorus and zinc, 12–14
minimum pasteurisation temperatures and times, 315
nutritional benefits, 5–15
organic milk improvement, 283–300
factors affecting the quality, 284–6
future trends, 299–300
management and husbandry techniques, 286–99
present phosphorus forms, 212
preventing microbial spoilage and chemical deterioration, 97–122
future trends, 122
pasteurised and ESL milk spoilage, 99–107
UHT and sterilised milk, 108–21
protection from obesity, 44–50
calcium, 44–7
conjugated linoleic acid, 47–8
effect of 300 mg increment on regular calcium intake, 46
effects of fatty acids and season on body weight, 48
effects of milk protein and appetite-regulatory bioactive peptides, 49
energy density of selected foods, 45
medium-chain fatty acids, 49
milk and obesity, 44
milk as a fat burner, 49–50
quality requirements for cheesemaking, 433–46
effects on yield and quality, 435, 437–42
future trends, 446
influence of milk preparation, 442–6
range of milk used in cheesemaking, 434–5
quality requirements for yoghurt-making, 417–29
base milk, 419–23
establishing conditions for coagulation, 423–4
factors that affect coagulation, 426–8
final steps in the process, 428–9
future trends, 429
yoghurt coagulum formation and structure, 424–6
role in diet, 3–19
salts partition in cow's milk, 208
sensory evaluation, 159–74
advanced statistical methods application, 172–4
historical perspective, 160–1
key issues, 159–60
methods, their application and effectiveness, 161–72

© Woodhead Publishing Limited, 2010

principles, 161
sheep milk quality and safety improvement, 347–81
developments in processing techniques, 381
factors affecting the quality, 349–52
historical perspective, 347–8
improving production, 359–74
management factors affecting the quality, 354–9
milking ewes management, 374–81
physiological factors affecting the quality, 352–4
processing, 348–9
sources of hazards for different products, 312
vitamins, 14–15
fat-soluble, 14–15
water-soluble, 14
world-wide consumption, 4–5
milk basic protein, 35
milk carbohydrate, 10–11
milk colour
and flavour, 182–8
future trends, 198
measurement, 197–8
instrumental, 181–98
milk components
applications in products other than foods, 475–84
non-food uses, 476–83
lactose and whey fermentations, 481–3
milk fat, 483
milk proteins, 476–81
technical applications, 477
milk ejection reflex, 354, 355
milk fat, 7–10, 484
non-food uses, 483
milk fat globule membrane protein
human milk, 456
postulated bioactivity, 457
milk flavour
and colour, 182–8
headspace methods, 189–91
dynamic headspace analysis, 190
static headspace analysis, 189–90
static headspace-solid phase microextraction, 191
instrumental methods of analysis, 181–98
future trends, 198
gas chromatography-mass spectrometry, 193–5
gas chromatography-olfactometry, 195
multivariate analysis and electronic noses, 196–7
quantitative analysis, 195–6
measurement, 188–97
solvent extraction-distillation methods, 191–3
class fractionation and concentration of extracts, 192–3
direct solvent extraction, 191–2
high vacuum distillation, 192
milk gel, 328
milk letdown, 408
milk powder, 406
milk protein, 6–7, 477
and peptide components, 75–9
$\alpha$-lactalbumin, 77–8
$\beta$-lactoglobulin, 76–7
bioactive peptides, 78–9
immunoglobulins, 76
lactoferrin, 76
lactoperoxidase, 76
hydrolysates, 85, 466–9
bioactive peptides, 466–8
bitterness reduction, 468
increased rate of uptake, 466
tolerogenic peptides, 468
use of other protein sources, 469
milk proteins, 42
non-food uses, 476–81
biodegradable materials, 476, 478–80
other non-food uses, 480–1
oxygen permeabilities and tensile properties of protein-based films and synthetic films, 479
polymorphism, 332
protection from obesity, 49
Milk Quality Improvement Program, 165
milk stone, 480
milk urea, 368
milking machine, 261
mineral content
analysis and improvement in milk, 207–21
increase in milk, 215–20
calcium addition, 215–17
calcium-chelatants addition, 219
orthophosphate addition, 217–18
sodium chloride addition, 217
ion exchange chromatographies, 220–1
membrane technologies
increase of mineral content in milk, 219–20
reduction of mineral content in milk, 220
methods for analysis, 210–15
mineral quantifications, 211–14
phosphorus forms present in milk, 212
preparation of sample, 210–11
salt equilibria theoretical calculation, 215
minerals of milk, 207–10
aqueous, 208
ions and salts concentrations in aqueous phase, 208
micellar phase, 208–9
salts partition in cow's milk, 208

© Woodhead Publishing Limited, 2010

salt equilibria, 209–10
between aqueous and micellar phases, 209
mineral quantifications, 211–14
catalase enzyme electrode, 213
fluorimetric methods, 213
ionic chromatography and capillary electrophoresis, 213–14
selective electrodes, 213
spectrophotometric methods
calcium, 213
chloride, 214
citrate, 214
phosphorus, 214
titration
calcium and magnesium, 212–13
chloride, 214
minerals, 80–1
minimal processing techniques, 462
Minnesota Farmstead, 87
mixed grass, 287
MMV process, 324–5
technique, 325
molasses, 409
molybdenum, 299
Monterey Jack goat milk cheese, 311, 325
mould, 377
Mozzarella cheese, 325, 327, 407, 438
MTGase *see* microbial transglutaminase
MucoVax, 83
Munsell colour system, 197
Murciana-Granadina, 334
Murrah, 408
Murray Goulburn, 273, 275
*Mycobacterium paratuberculosis*, 438
mycotoxins, 377

N-acetyl-$\delta$-glucosamine, 461
Nachtmilch, 88
nanofiltration, 264, 273
naphthalene, 372
National Advisory Committee on Microbiological Criteria for Foods, 310
National Conference on Interstate Milk Shipments, 306
National Mastitis Council, 321
National Sustainable Agriculture Information Service, 255
NDF *see* neutral detergent fibre
Nestlé, 83, 267
neutral detergent fibre, 359, 363, 364
NFC *see* non-fibrous carbohydrates
NHANES I Epidemiologic Follow-up Study, 8
niacin, 14
*see also* nicotinic acid
niacinamide, 239–40
nicotinamide, 240
nicotinic acid, 239
night-time milk, 88
nitrous oxide, 260, 261
non-fibrous carbohydrates, 359, 363
non-protein nitrogen, 434
human milk, 459
non-starter lactic acid bacteria, 437
non-structural carbohydrate, 365
Norwegian Reds, 299
NPN *see* non-protein nitrogen
NSC *see* non-structural carbohydrate
NSLAB *see* non-starter lactic acid bacteria
Nubian breed, 318
nucleosides, 459
Nurses' Health Study, 7
nutrition
fat supplements
rumen-protected, 365
rumen-unprotected, 365–7
for sheep milk production improvement, 361, 363–73
alternative feed sources, 369–70
dietary fibre, 364
dietary protein, 367–8
energy balance and milk fat and protein content, 363–4
fat supplements, 364–5
milk fat depression syndrome in sheep, 367
pasture, 370–3

Oberhasli, 318
obesity, 15–16
ochratoxin A, 378
ochratoxin-$\alpha$, 378
off-flavours, 147
in fluid and dried milks, 186–7
ohmic heating, 107
oligopeptides, 464
oligosaccharides, 80, 463
human milk, 457–8
olive cake, 369
on-column injection, 194
opsonisation, 458
oral tolerance, 468
organic acids, 484
organic milk
factors affecting the quality, 284–6
implications of meeting the commercial market requirements, 285
limitations on individual dairy farm, 285–6
organic standards limitations, 284–5
future trends, 299–300
grazing strategies and role of white clover, 291–4
buffer feeding as a management option during grazing season, 294
influence of stocking density and type of grazing system, 293

© Woodhead Publishing Limited, 2010

protein changes in the composition of organic swards, 292
improvement, 283–300
management and husbandry techniques, 286–99
genetics compatible with organic systems, 298–9
nutritional balance of diets, 286–7
optimising feed quality with cow's peak nutrient requirements, 294–5
minimising health risk and welfare problems, 295–7
metabolic disorders, 296–7
reducing stress and behavioural problems, 297
somatic cell counts, 295–6
organic forage cropping options, 287–91
higher energy forages, 289
higher protein forages, 288–9
permanent pastures important role, 290
reseeded leys quality improvement, 289–90
silage quality improvement on organic farms, 290–1
Organic Valley Pasture Butter, 87
orotic acid, 459
orthophosphate, 217–18
osteoporosis
and calcium, 29–30
prevention and bone health, 35–6
ovine milk, 425–6
oxidised flavour, 307
oxygen, 148
oxygen-barrier polymer, 480
oxytocin, 354, 355, 356

*P. fluorescens*, 439
packaging
effects on milk quality and safety, 136–55
environmental issues, 151–5
incineration and sanitary landfilling, 154–5
recycling, 153–4
reuse-multiple trip containers, 152–3
factors affecting milk shelf-life and safety, 145–9
light, 146–8
microorganisms and temperature, 146
oxygen, 148
permeability to flavours, 149
water vapour permeability, 148–9
migration and flavour scalping, 149–51
flavour scalping, 151
migration, 149–50
testing conditions for migration testing, 151
primary packaging materials, 137–42
all-plastic containers, 139
aluminium cans, 138
fibreboard cans/composites, 142
glass bottles, 138–9
HDPE jugs, 139–40
HIPS, 140–1
LLDPE/LDPE pouches, 141
paperboard based containers, 141–2
PET bottles, 140
polycarbonate bottles, 140
tin cans, 137–8
secondary and tertiary packaging materials, 142–5
crates and drums, 144
fibreboard-corrugated board, 142–3
films, 143–4
paper sacks, 145
paperboard composites, 143
plastics, 143
sacks, 145
types and their applications, 137–45
PaddockGRASP, 255
*Paenibacillus polymyxa*, 100
pantothenic acid, 240–1
paper, 153–4
paper sacks, 145
Parmesan cheese, 351
pasteurisation, 307, 314–15, 322–3
contamination prevention guidelines, 315–16
guidelines, 314–15
principle, 314
pasteurised milk
potent odourants identified by GCO, 183
spoilage, 99–107
PBS *see* polybutylene succinate
PBT *see* polybutylene terephthalate
PCA *see* principal component analysis
PCBs *see* polychlorinated biphenyls
PCDD *see* polychlorinated dibenzodioxins
PCDFs *see* polychlorinated dibenzofurans
PCR-AS, 334
PCR-RFLP, 334
PDO *see* protection of origin
peanut, 368
Pecorino, 407
PEF *see* pulsed electric fields
pellagra, 240
penicillin, 426
*Penicillium*, 378
peptides, 39
perennial ryegrass, 288
peroxidases, 479
pesticides, 381
PET *see* polyethylene terephthalate
PET bottles, 140
phe-met, 444
phenol, 372
phosphatidylinositol, 41
phospholipids, 404, 463
phosphorus, 14, 404, 406
forms present in milk, 212

© Woodhead Publishing Limited, 2010

quantification by spectrophotometric method, 214
phthalate, 381
phylloquinone, 236–7
*Pisum sativum*, 369
PLA *see* polylactic acid
plasmin, 109, 439, 440
vs proteolytic enzymes of psychotrophic bacteria, 111–12
plasminogen, 354, 439
plasticisers, 478
plastics, 143, 153–4
plate heat exchanger, 423
Poitevine, 334
polar lipids, 80
Poll Dorset, 350
poly unsaturated fatty acid, 337, 365
polyamides, 480
polybutylene succinate, 478
polybutylene terephthalate, 478
polychlorinated biphenyls, 380
polychlorinated dibenzodioxins, 380
polychlorinated dibenzofurans, 380
polyethylene, 480
polyethylene terephthalate, 139, 140, 480
polylactic acid, 478
polypropylene, 480
polysaccharides, 328
polytrimethylene terephthalate, 478
polyunsaturated fatty acids, 469
polyvinylidene chloride, 480
potassium, 12–13
potent growth factor activity, 458
pre-menstrual syndrome, 18
Préalpes ewes, 355
prebiotics, 469
effects on gut health, 50–60
gut associated health effects, 57
safety, 57–8
principal component analysis, 173
probiotics, 418, 424, 425, 427, 462, 469
effects on gut health, 50–60
evidence for beneficial effects
on cancer, 56
on gastrointestinal infections and diarrhoea, 54–5
on lactose intolerance, 53–4
health effects, 52
health effects and mechanisms, 52–6
constipation, 55
IBD and IBS, 55–6
improving milk products for gut health, 58–9
safety, 57–8
programmable temperature vaporiser, 194
prolactin, 354
proline-specific enzyme, 468
proteases, 112
protection of origin, 442–3
protein, 349, 404
proteinases, 100
proteolysis, 296, 445
proteolytic enzymes, 111
*Pseudomonas*, 111, 352, 439
*Pseudomonas fluorescens*, 122
*Pseudomonas* spp, 423
PTT *see* polytrimethylene terephthalate
Public Health Service, 307
pulsed electric fields, 462
putrescine, 459
PVDC *see* polyvinylidene chloride
pyridoxine, 241–2
pyroxidal-5′-phosphate, 241

quantitative descriptive analysis, 170
quantitative trait loci, 359
Quark, 433
quorum sensing, 122

Rambouillet ewe, 353
rancid, 306, 307
raw milk
potent odourants identified by GCO, 183
psychotrophic bacterial growth, 117
recycling, 153–4, 275
aluminium, 153
glass, 153
plastics and paper, 153–4
tinplate, 153
red clover, 287, 288, 440
Red Sokoto, 318
refrigeration, 266–8
rennet, 433, 444, 445
rennet cheese, 325
rennet clotting time, 407, 445
rennet coagulation, 334, 441
rennet curds, 379–80
retentate, 444
reverse osmosis, 273
reversed-phase HPLC, 460
riboflavin, 14, 102, 238–9, 323, 406
Ricotta, 407
river buffalo, 403
roller drying process, 330
Rosenholm Farm, 257
rotational grazing, 255, 257
financial savings, 256
Rumentek, 86
ruminal biohydrogenation, 338
ryegrass, 289, 369, 372

Saanen breed, 317–18, 332, 334
*Saccharomyces* spp, 423
sacks, 145
SAFE *see* solvent assisted flavour evaporation
Saint-Nectaire cheeses, 441
salic acid, 459
*Salmonella enterica*, 437
*Salmonella* spp, 352, 423

© Woodhead Publishing Limited, 2010

*Salmonella* Typhimurium, 438
salt equilibria, 209–10
  between aqueous and micellar phases, 209
  theoretical calculation, 215
sanitary landfilling, 154–5
Sarda breed, 351, 353, 356, 361, 365, 369, 370, 376
SCC *see* somatic cell count
SCD gene *see* stearoyl Co-A gene
score card, 160
SDFe *see* superdispersed ferric pyrophosphate iron
selected ion monitoring mass spectrometry, 194
selective hydrolysis, 465
selenium, 81, 87
selenium deficiency, 373
selenium-enriched milk, 87
Senrich bolus, 87
sensory evaluation, 159–74
  advanced statistical methods application, 172–4
  definition, 161
  historical perspective, 160–1
  key issues, 159–60
  methods, their application and effectiveness, 161–72
    acceptance and preference tests, 163–72
    ballot used for ultra-pasteurised milk shelf-life studies, 167–9
    descriptive analysis, 163
    discrimination tests, 162–3
    hedonic scales, 164
    intensity line scales, 171
    reduced fat milk sensory profiles, 165
  principles, 161
set yoghurt, 418, 419
sheep milk
  aflatoxin M1 concentrations, 379
  daily energy balance and milk fat content relationship, 363
  developments in processing techniques, 381
  effects of free oil supplements, 366
  factors affecting the quality, 349–52
    genetic factors, 349–51
    microbial cell count, 352
    somatic cell count, 351–2
  fatty acid composition, 338
  food intake, milk yield and composition, efficiency feeding index and feeding costs, 371
  historical perspective, 347–8
  improving production, 359–74
    genetic approach, 359–61
    non-genetic approach, 361
    nutrition, 361, 363–73
    nutritional imbalances and SCC, 373–4
  lactation curves of dairy ewes, 377
  management factors affecting the quality, 354–9
    environmental conditions and milk composition, 356–7
    milking interval and frequency, 354–5
    milking techniques, 354
    nutrition, 359
    stripping method, 355–6
  management of the ewes, 357–9
    artificial lighting/photoperiod, 358
    breeding out of season, 357–8
    shearing, 357
    use of hormones, 359
  milk fat concentration and yield, 362
  milk protein concentration and yield, 362
  milk urea N concentration and percentage of dietary crude protein, 368
  milking ewes management, 374–81
    contaminants in sheep milk, 377–81
    mastitis, 376–7
    milking operations, 374–5
    seasonality and breeding out of season, 375–6
  NDF dietary content and milk fat content relationship, 364
  physiological factors affecting the quality, 352–4
    age and parity, 352
    lactation stage, 353
    number of lambs born or weaned, 353–4
    weight of ewes, 353
  processing, 348–9
  protein and fat concentrations in different sheep breeds, 350
  QTL reported for dairy sheep, 360
  quality and safety improvement, 347–81
  seasonal evolution of *cis*-9, *trans*-11 CLA, 373
Shorthorn, 298
shrinkability, 143, 144
SIDA *see* stable isotope dilution analysis
silage, 290
simultaneous distillation-solvent extraction, 191
skatole, 369
skim milk, 328
skim-milk powder, 421
Slow Food, 381
Slumber Bedtime milk, 88
sodium, 213
sodium caseinate, 328
sodium chloride, 217
soft cheese, 327
soil characteristics, on dairy farming, 253–7
  soil and river bank erosion, 255–6
  soil compaction, 254
  soil fertility loss, 256
solids-non-fat, 318, 419

© Woodhead Publishing Limited, 2010

solvent assisted flavour evaporation, 192
somatic cell count, 296, 306, 336–7, 349, 361, 373, 439
sour cream, 327
soy protein, 328
soybean hulls, 369
soybean meal, 368, 369
soybean oil, 365
spectrophotometric methods, 213, 214
spermidine, 459
spermine, 459
sphingolipids, 483
sphingomyelin, 41
spoilage enzymes
  impact on quality, 109–14
  lipolytic, 112–14
  proteolytic, 109–12
    gelation, 109
    heat-resistant endogenous protease plasmin, 109, 111
    plasmin vs proteolytic enzymes of psychotrophic bacteria, 111–12
    proteolysis, 109
spontaneous lipolysis, 322
spray drying process, 330
St. Paulin cheese, 325
stabilisers, 328
stable isotope dilution analysis, 196
*Staphylococcus aureus*, 296, 437, 438
*Staphylococcus uberis*, 296
starch, 459
static headspace analysis, 189–90
stearoyl Co-A gene, 360
sterilisation, 99
sterilised milk, 108–21
stirred yoghurt, 418, 419, 423
stocking density, 293
*Streptococcus* spp, 352
*Streptococcus thermophilus*, 327, 329, 418, 423, 424, 426, 427
streptomycin, 426
*Streptoverticicillium mobaranese*, 329
strip grazing, 297
stroke, 17
sucrose, 459
sunflower oil, 365
sunflower seeds, 367
superdispersed ferric pyrophosphate iron, 326
supply chain management, 274
sustainable farming, 253–4
swamp buffalo, 403, 409
Swedish Red, 299
Swiss Red, 298
*Syncerus*, 403
syndrome x *see* metabolic syndrome

taints, 427
tannic acid, 479
tannins, 369
themisation, 439
thiamin, 14, 237–8, 323
threonine, 422
thresholds, 166
timothy grass, 288, 289–90
tin cans, 137–8
tinplate, 153
titration, 212–13, 214
tocopherol, 102
Toggenburg, 318
toluene, 372
toned milk, 407
total utilisable substances, 370
toxic metals, 379
*trans*-10, *cis*-12 CLA, 367
transgalactosylation reaction, 482
transglutaminases, 479
triangle test, 162
trichothecenes, 378
trimethylamine, 100
tryptophan, 14, 77

UHT *see* ultra-high temperature treatment
UHT cream, 406
UHT milk, 422
  factors influencing spoilage, 115–18
    dairy cold chain, 116
    factors influencing stability, 117–18
    highly heat-resistant spore formers: role of farm and dairy, 115–16
  potent odourants identified by GCO, 183
  processing, 98
  spoilage, 108–21
ultra-high temperature treatment, 323
ultrafiltration, 273, 433
  buffalo milk, 407
  goat milk, 324–5
  hypoallergenic infant formula production, 465
  influence in cheesemaking properties and cheese quality, 444–5, 446
  reduction of bitterness, 468
  yoghurt-making standardisation, 420
unsaturated fat, 85–6
urea, 292, 409, 459
uric acid, 459

van der Waal bond, 477
vat pasteurisation, 315
vetch, 287, 288
*Vicia faba*, 369
vitamin A, 14–15, 102, 231–3, 291, 326, 406
vitamin $B_1$ *see* thiamin
vitamin $B_2$ *see* riboflavin
vitamin $B_3$ *see* niacinamide
vitamin $B_5$ *see* pantothenic acid
vitamin $B_6$ *see* pyridoxine
vitamin $B_8$ *see* biotin
vitamin $B_9$ *see* folates

© Woodhead Publishing Limited, 2010

vitamin $B_{12}$ *see* cyanocobalamin
vitamin B complex, 406
vitamin $B_{12}$ deficiency, 245
vitamin C, 245–6, 323, 406
  *see also* ascorbic acid
vitamin D, 31, 233–5, 326
vitamin E, 121, 235–6, 291
vitamin $K_1$ *see* phylloquinone
vitamin $K_3$ *see* menadione
vitamin K deficiency, 237
vitamins, 14–15, 484
  analysing and improving levels in milk, 229–48
  fat-soluble, 14–15, 230
  group B vitamins, 237–45
  hydrophilic, 237–46
  lipophilic, 231–7
  liposoluble and hydrosoluble vitamins and pseudovitamins, 232
  naturally occurring in cow's milk, 231–46
  techniques to improve content in milk, 246–7
  water-soluble, 14, 230
  *see also* specific vitamin

waste minimisation triangle, 274
water, in dairy processing
  boilers and cooling towers, 272–3
  cleaning, 272–3
  leaks and process control, 271
  recycling and reuse, 273
Water Account database, 270
water recirculation system, 273
water-soluble vitamins, 14
water vapour permeability, 148–9
Welsh, 351
West African Dwarf, 318
whey, 273, 275
whey fermentations
  non-food uses, 481–3
whey products, 328
whey protein/casein ratio, 433
whey protein coatings, 480
whey protein concentrates, 404
whey proteins, 353, 406, 463, 477
  denatured, 428
  powder, 421
White breeds, 298
white clover, 287, 288, 289, 294
white clover swards, 300
WHO Technical Report Series 916, 5
whole-crop cereal silage, 289
wild camomile, 427
wild flavour, 446
Wisconsin dairy goat cooperative, 323
Wisconsin Mastitis Test, 306

xanthan gum, 483

yellow index, 197
*Yersinia*, 322
yoghurt, 327, 406, 418, 429
yoghurt gel, 421
yoghurt-making
  advantage of homogenisation, 421–2
  base milk, 419–23
    essential components from bovine milk, 419–20
    heat treatment effect, 422–3
    homogenisation effect, 421–2
    standardisation, 420–1
  coagulum formation and structure, 424–6
  establishing conditions for coagulation, 423–4
  factors that affect coagulation, 426–8
    extraneous materials in milk, 426–7
    natural changes in milk quality, 427–8
  final steps in the process, 428–9
  future trends, 429
  goat milk yoghurt-processing, 327–9
    texture enhancement by ingredient fortification, 328
    texture improvement by enzymatic crosslinking, 329
  milk quality requirements, 417–29
  typical modern process, 418–19
  yoghurt milks chemical composition, 421

zearalenone, 378
zeta potential, 421
zinc, 13–14

© Woodhead Publishing Limited, 2010