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Quality Attributes and Consumer Values

Edited by Massimiliano Petracci and Cécile Berri

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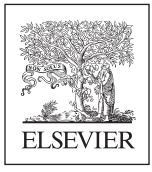
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Edited by

Massimiliano Petracci
University of Bologna, Bologna, Italy

Cécile Berri
URA, INRA, Nouzilly, France



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List of Contributors

Shai Barbut University of Guelph, Guelph, ON, Canada

Cécile Berri URA, INRA, Nouzilly, France

Alessandra Bordoni University of Bologna, Bologna, Italy

Brian Bowker United States Department of Agriculture, Athens, GA, United States

Francesco Capozzi University of Bologna, Cesena, Italy

Rafael Carvalho State University of Londrina, Londrina, Brazil

Cesare Castellini University of Perugia, Perugia, Italy

Marianne Chemaly French Agency for Food, Environmental and Occupational Health Safety (Anses), Ploufragan, France

Daniel Clark The Ohio State University, Wooster, OH, United States

Alessandro Dal Bosco University of Perugia, Perugia, Italy

Francesca Danesi University of Bologna, Bologna, Italy

Alessandra De Cesare University of Bologna, Bologna, Italy

Mario Estévez University of Extremadura, Cáceres, Spain

Pasquale Ferranti University of Naples "Federico II", Portici, Italy

Muriel Guyard French Agency for Food, Environmental and Occupational Health Safety (Anses), Ploufragan, France

Rachel Harding The Ohio State University, Wooster, OH, United States

Pedro C. Hernandez Institute of the Structure of Matter (CSIC), Madrid, Spain

Ana M. Herrero Institute of Food Science, Technology and Nutrition (ICTAN-CSIC), Madrid, Spain

Francisco Jiménez-Colmenero Institute of Food Science, Technology and Nutrition (ICTAN-CSIC), Madrid, Spain

Elisabeth Le Bihan-Duval URA, INRA, Nouzilly, France

Gerardo Manfreda University of Bologna, Bologna, Italy

Marine Meunier French Agency for Food, Environmental and Occupational Health Safety (Anses), Ploufragan, France

Amit Morey Auburn University, Auburn, AL, United States

Casey M. Owens University of Arkansas, Fayetteville, AR, United States

Patricia Parrott Harper Adams University, Newport, United Kingdom

Frederique Pasquali University of Bologna, Bologna, Italy

Claudia R.-C. Perez Institute of Food Science, Technology and Nutrition (ICTAN-CSIC), Madrid, Spain

Massimiliano Petracci University of Bologna, Bologna, Italy

Katell Rivoal French Agency for Food, Environmental and Occupational Health Safety (Anses), Ploufragan, France

Massami Shimokomaki State University of Londrina, Londrina, Brazil

Francesca Soglia University of Bologna, Bologna, Italy

Alessia Trimigno University of Bologna, Cesena, Italy

Keith Walley Harper Adams University, Newport, United Kingdom

Hans-Wilhelm Windhorst University of Vechta, Vechta, Germany

Preface

Poultry, and especially chicken meat, will overtake pork in very few years as the most popular meat being consumed in the world. The main reasons for this popularity are ascribable to its being the cheapest and most accessible meat in the world, as well as to the absence of any sort of cultural barriers such as those that affect pork and beef meat. A healthier profile and a suitability for being used for manufacturing attractive, convenient, easy to use processed products also make poultry the preferred meat in modern and multicultural societies where people tend to live and work in urban areas, taking most meals away from home and spending less and less time on home meal preparation, together with increasing culinary ignorance. Finally, in a situation in which sustainability implications associated with meat consumption cannot be ignored, the poultry industry is generally recognized as being much kinder to the environment than that of almost every other kind of meat.

These advances have been made possible thanks to the progress in genetics, nutrition and feeding, physiology, farming management, veterinary medicine, automation, and so on. However, as already mentioned, society's perception of animal production systems and ways of consuming meat and meat products are changing very rapidly, therefore the challenge for the poultry industry is not to lose sight of the ultimate goal of the entire poultry chain: to continue to produce products that are in line with the expectations of society and consumers. That said, it is amazing that worldwide there are few research works dedicated to the quality of poultry meat, unlike in the mammalian meat sector, where there are well-recognized scientific societies, specialized journals, and congresses.

For this reason, the need for updated and advanced books on the quality of poultry meat is therefore particularly felt by students, professors, scientists, technicians, industry people, and professionals. But at the same time, it is not easy to identify key researchers with the necessary skills and experience who are able to handle not only more classical topics, but also frontier ones. Therefore, following stimulation given by the publisher, we decided to split the book into three parts: the first part is dedicated to the association between muscle structure and the basic qualities of eating cooked meat, while the second deals with new techniques for measuring and predicting meat quality. The final part covers the current qualities of consumer and public perceptions regarding sustainable, ethical, and health issues.

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Chapter 1

Dynamics and Patterns of Global Poultry-Meat Production

Hans-Wilhelm Windhorst
University of Vechta, Vechta, Germany

1.1 INTRODUCTION

Of all animal products, poultry meat showed the highest absolute and relative growth rates between 1994 and 2014. This not only demonstrates the important role that poultry meat plays as a protein source for a growing global population, but also the efficient production systems. In the first part of this analysis, the dynamics of poultry-meat production on various spatial levels will be analyzed; in the second part, an overview on the projected development of production and consumption until 2024 will be presented.

1.2 THE DYNAMICS OF GLOBAL POULTRY-MEAT PRODUCTION BETWEEN 1994 AND 2014

Between 1994 and 2014, the global production volume of poultry meat increased by 62.0 million tons or 121.8%. To this growth, chicken meat contributed 56.7 million tons or 91.4%. All other meat types showed much lower absolute growth rates, the lowest increase was to be found in turkey meat with only 1.4 million tons or 32.0% (Table 1.1).

A closer look at the dynamics at the continent level (see also Windhorst, 2014a, 2014b, 2014c; Windhorst and Wilke, 2015) reveals that the absolute growth of poultry-meat production was highest in Asia with 25.0 million tons followed by Central and South America with 14.9 million tons. These two continents shared 64.3% of the global growth. In Europe and North America, the relative increase of the production volume was much lower than in the other continents, a result of the already high production in 1994 (Table 1.2). The changing contribution of the continents to global poultry-meat production in the analyzed time period is shown in Fig. 1.1. One can easily see that the contribution of Asia and Central and South America

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TABLE 1.1 The Development of Global Poultry-Meat Production Between 1994 and 2014 by Meat Type (Data in 1000 t)

Meat type	1994	2014	Increase (1000 t)	Increase (%)
Chicken meat	43,688	100,353	56,665	129.7
Turkey meat	4252	5611	1359	32.0
Duck meat	1894	4331	2437	128.7
Others	1083	2638	1555	143.6
Total	50,917	112,933	62,016	121.8

Source: FAO database. faostat.fao.org. (data of January 16th, 2017).

TABLE 1.2 The Development of Global Poultry-Meat Production Between 1994 and 2014 at Continent Base (data in 1000 t)

Continents	1994	2014	Increase (1000 t)	Increase (%)
Africa	2156	5685	3259	163.7
Asia	15,497	40,504	25,007	161.4
Europe	10,716	19,233	8517	79.5
North America[a]	15,263	24,587	9324	61.1
Central and South America	6704	21,569	14,865	221.7
Oceania	581	1355	774	133.2
World	50,917	112,933	62,016	121.8

[a]*Canada, Mexico, USA.*
Source: FAO database. faostat.fao.org. (data of January 16th, 2017).

increased considerably while Europe and North America lost 4.0% and 8.2%, respectively, of their former share.

The analysis of the dynamics of poultry-meat production on country development base (see Windhorst, 2015) shows that the production volume of the newly industrialized countries (NICs) (or threshold countries) grew by 32.1 million tons. They contributed 51.8% to the global increase, mainly a result of the extraordinary development in China and Brazil. Despite the highest relative growth, the least-developed countries were only able to expand their production by 2.2 million tons (Table 1.3). Fig. 1.2 shows that

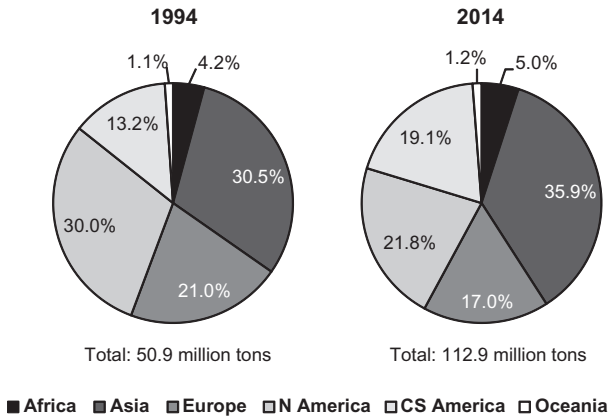


FIGURE 1.1 The changing contribution of the continents to global poultry-meat production between 1994 and 2014. *Own design.*

TABLE 1.3 The Development of Global Poultry-Meat Production Between 1994 and 2014 at Country Development Level (data in 1000 t)

CDG	1994	2014	Increase (1000 t)	Increase (%)
LLDCs	724	2902	2196	300.8
LDCs	8110	21,057	12,947	159.6
NICs	16,648	48,797	32,149	193.1
OICs	25,435	40,177	14,742	58.0
World	50,917	112,933	62,016[a]	121.8

CDG, country development group; *LLDCs*, least-developed countries; *LDCs*, less-developed countries; *NICs*, newly industrialized countries; *OICs*, old industrialized countries.
[a]*Sum does not add up because of rounding.*
Source: FAO database. faostat.fao.org. (data of January 16th, 2017).

the share of the NICs increased by 10.5% between 1994 and 2014, while that of the old industrialized countries decreased by 14.2%.

From Fig. 1.3 one can easily see that global poultry-meat production in 2014 was concentrated in four main clusters (North America, South America, the European Union, and East Asia). Sub-centers were to be found in Northern Africa, West Asia, South and Southeast Asia. The spatial pattern reflects the differences in the per capita consumption (see also Table 1.19 and Fig. 1.4), the distribution of the global population as well as the development status of the countries and the income situation of the population. The

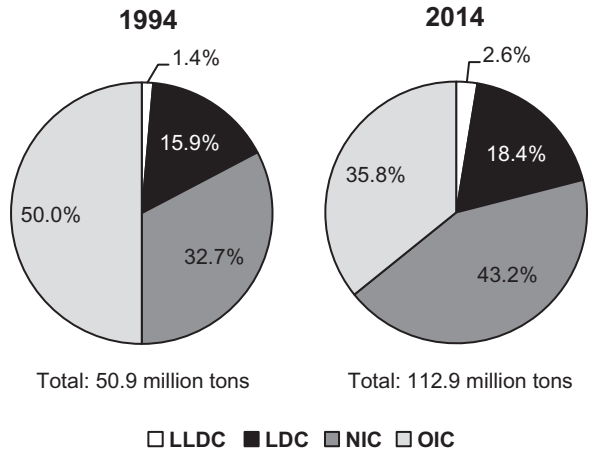


FIGURE 1.2 The changing contribution of the country development groups to global poultry-meat production between 1994 and 2014. *Own design.*

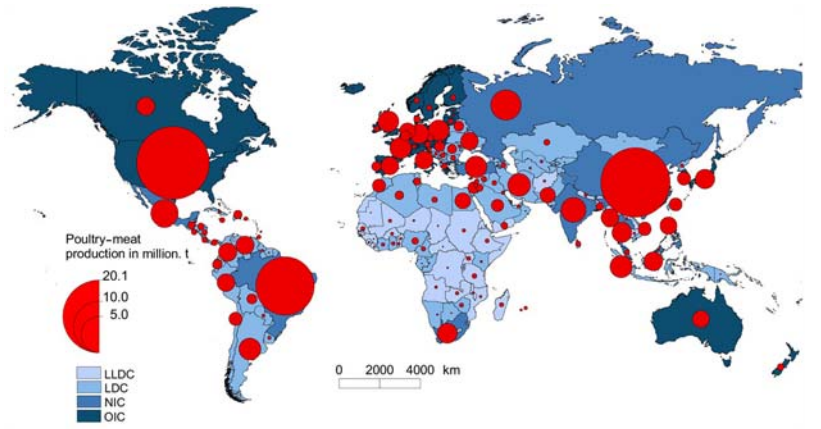


FIGURE 1.3 The spatial distribution of poultry-meat production on the basis of country development groups in 2014. *Own design.*

low production volumes in the countries in Central Asia as well as in Sub-Saharan Africa, with the exception of South Africa, are obvious. In Sub-Saharan Africa, the low development status, lack of capital and know how as well as the instability of the political systems in many countries are the main steering factors behind this situation.

A comparison of the composition and ranking of the 10 leading countries in poultry production in 1994 and 2014 demonstrates the leading roles of the USA, China, and Brazil (Table 1.4). These three countries contributed 46.5% to the global production volume in 1994 and 45.6% in 2014. While the USA lost 8.8% of their former share, China gained 3.3%, and Brazil 4.6%. The

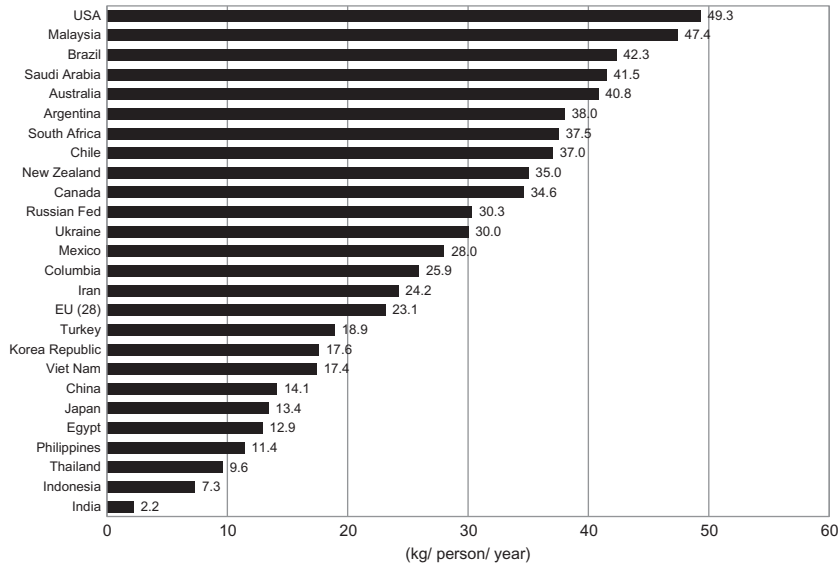


FIGURE 1.4 Projected per capita consumption of poultry meat in selected countries in 2024. *From OECD-FAO Agricultural Outlook 2015. OECD Publishing, Paris. http://dx.doi.org/10.1787/agr-outlook-2015-en, own design.*

absolute increase was highest in China with 11.7 million tons, followed by Brazil with 9.5 million tons and the USA with 7.1 million tons. It is worth mentioning that the gap between the production volumes in the USA and Brazil, the two leading exporting countries, became smaller, but was still as wide as 7.4 million tons in 2014. The regional concentration, i.e., the share of the 10 countries in global production, fell from 63.2% to 61.3%, an indicator that poultry-meat production also grew considerably in other than the 10 listed countries. It is also worth mentioning that India, Iran, Indonesia, and Argentina were listed among the 10 leading countries in 2014 while France, the United Kingdom, Italy, and Thailand lost their leading positions which they had occupied in 1994. This again demonstrates that the fastest growth of poultry-meat production occurred outside the EU and North America.

1.3 THE PATTERNS OF POULTRY-MEAT PRODUCTION IN THE COUNTRY DEVELOPMENT GROUPS IN 2014

In a second step, the patterns of poultry-meat production in the four country development groups, as distinguished in this paragraph (for the classification of the countries see Windhorst, 2015), will be analyzed in more detail.

Table 1.5 shows that of the 208 countries for which data on poultry meat production is available, 48 belonged to the group of the least-developed

TABLE 1.4 The 10 Leading Poultry-Meat-Producing Countries in 1994 and 2014 (Data in 1000 t)

1994			2014		
Country	Production	Share (%)	Country	Production	Share (%)
USA	13,247	26.8	USA	20,392	18.0
China	6500	12.8	China	18,197	16.1
Brazil	3512	6.9	Brazil	12,997	11.5
France	1972	3.9	Russian Federation	3770	3.3
UK	1346	2.7	Mexico	2920	2.6
Japan	1256	2.5	India	2759	2.4
Mexico	1155	2.3	Japan	2128	1.9
Italy	1114	2.2	Iran	2081	1.8
Russian Federation	1068	2.1	Argentina	1979	1.8
Thailand	996	2.0	Indonesia	1977	1.8
10 countries	32,184	63.2	10 countries	69,189[a]	61.3[a]
World	50,917	100.0	World	112,933	100.0

[a]*Sum does not add up because of rounding.*
Source: FAO database. faostat.fao.org. (data of January 16th, 2017).

TABLE 1.5 The Share of the Country Development Groups in the Global Population and in Poultry-Meat Production in 2014 (Data in %)

CDG	Countries (number)	Population	Poultry-Meat Production
LLDCs	48	13.0	2.6
LDCs	113	24.2	18.4
NICs	10	48.0	43.2
OICs	37	14.8	35.8
Total	208	100.0	100.0

CDG, country development group; *LLDCs*, least-developed countries; *LDCs*, less-developed countries; *NICs*, newly industrialized countries; *OICs*, old industrialized countries.
Source: FAO database. faostat.fao.org. (data of January 16th, 2017), own calculations.

countries. They shared 13.0 % of the global population but only 2.6% in world poultry-meat production.

As can be seen from Table 1.6, the 10 leading countries of this group produced about 2.4 million tons of poultry meat in 2014. Myanmar with a production volume of 1.5 million tons contributed 52.7% to the overall production of this country group, followed by Bangladesh (7.7%), and Yemen (5.3%). Because of the dominating role of Myanmar, the regional concentration in this group was very high, for 81.7% of the total production was contributed by only 10 countries.

Of the analyzed countries, 113 or 54.3% belonged to the group of the less-developed countries. They shared 24.2% of the global population and 18.4% of world poultry-meat production. This was a relatively imbalanced situation. A closer look at the composition and ranking of the 10 leading countries in this group reveals that, in contrast to the least-developed countries, no single country could reach a dominating position. In contrast, eight of the 10 listed countries produced more than 1 million tons of poultry meat; Iran, Indonesia, and Argentina produced close to or even more than 2.0 million tons, Malaysia produced almost 1.6 million tons

TABLE 1.6 The 10 Leading Poultry-Meat-Producing Countries (2014) in the Group of Least-Developed Countries (Data in 1000 t)

Country	Production	Share (%) in CDG	Share (%) in Global Production
Myanmar	1.528	52.7	1.35
Bangladesh	223	7.7	0.20
Yemen	155	5.3	0.14
Malawi	86	3.0	0.08
Madagascar	72	2.5	0.06
Senegal	66	2.3	0.06
Uganda	65	2.2	0.06
Ethiopia	61	2.1	0.05
Sudan	58	2.0	0.05
Tanzania	56	1.9	0.05
10 countries	2,370	81.7	2.10
CDG LLDCs	2,902	100.0	2.60

CDG, country development group; *LLDCs*, least-developed countries.
Source: FAO database. faostat.fao.org. (data of January 16th, 2017).

TABLE 1.7 The 10 Leading Poultry-Meat-Producing Countries (2014) in the Group of Less-Developed Countries (Data in 1000 t)

Country	Production	Share (%) in CDG	Share (%) in Global Production
Iran	2081	10.0	1.8
Argentina	1979	9.5	1.8
Indonesia	1977	9.5	1.8
Malaysia	1571	7.6	1.4
Colombia	1359	6.6	1.2
Peru	1317	6.4	1.2
Ukraine	1167	5.6	1.0
Egypt	1165	5.6	1.0
Pakistan	992	4.8	0.0
Venezuela	886	4.3	0.8
10 countries	14,496	69.9	12.8[a]
CDG LDCs	20,725	100.0	18.4

CDG, country development group; *LDCs,* less-developed countries.
[a]*Sum does not add up because of rounding.*
Source: FAO database. faostat.fao.org. (data of January 16th, 2017).

(Table 1.7). The fact that the regional concentration was also much lower than in the group of the least-developed countries demonstrates the extraordinary role that poultry meat plays as a protein source for the population in these countries.

It was already mentioned that the newly industrialized or threshold countries showed the highest increase of the production volume of the four country development groups with 32.1 million tons between 1994 and 2014, sharing almost 52% in the global increase.

The 10 countries which belong to this group shared 48.0% of the global population and 43.2% of global poultry-meat production. The imbalance is a result of the high population figures in China and India and the comparatively low production and per capita consumption in India.

From the data in Table 1.8, one can see that the regional concentration in this country group was extremely high in 2014. China and Brazil, the two leading countries, contributed 63.9% of the overall production of this group and 27.6% of the global production volume. It is worth mentioning that the Russian Federation was able to expand its production volume by more than

TABLE 1.8 The 10 Leading Poultry-Meat-Producing Countries (2014) in the Group of Newly Industrialized Countries (Data in 1000 t)

Country	Production	Share (%) in CDG	Share (%) in Global Production
China	18,187	37.3	16.1
Brazil	12,997	26.6	11.5
Russian Federation	3770	7.7	3.3
Mexico	2920	6.0	2.6
India	2759	5.7	2.4
Turkey	1908	3.9	1.7
Thailand	1814	3.7	1.6
South Africa	1724	3.5	1.5
Malaysia	1573	3.2	1.4
Philippines	1147	2.4	1.0
CDG NICs	[a]48,797	100.0	43.2[a]

CDG, country development group; *NICs*, newly industrialized countries.
[a]*Sum does not add up because of rounding.*
Source: FAO database. faostat.fao.org. (data of January 16th, 2017).

2.2 million tons in the analyzed time period, a result of the high investments in this sector of animal production. It was much easier to install efficient broiler growing systems than to build large pork-producing complexes. Another country with a very dynamic development in broiler meat production is Turkey. The growth is also a result of a rapidly increasing domestic demand and of a growing export volume.

The 37 countries which are classified as old industrialized countries in this analysis shared 14.8% of the global population but 35.8% of global poultry-meat production. This imbalance is a result of the intensity of the production systems, the domestic demand in these countries because of the high per capita consumption and the important role which several of these countries plays in global poultry-meat exports.

A more detailed analysis of the data in Table 1.9 reveals the dominating role of the USA in this group, but also that all of the following nine countries produced more than 1 million tons of poultry meat in 2014. Six of them are members of the EU, two are located in North America, one in Asia, and one in Oceania. Because of the extraordinarily high production volume of the USA, the 10 leading countries contributed 85.6% of the overall

TABLE 1.9 The 10 Leading Poultry-Meat-Producing Countries (2014) in the Group of the Old Industrialized Countries (Data in 1000 t)

Country	Production	Share (%) in CDG	Share (%) in Global Production
USA	20,392	50.3	18.0
Japan	2128	5.3	1.9
Poland	1849	4.6	1.6
France	1767	4.4	1.6
UK	1648	4.1	1.5
Germany	1550	3.8	1.4
Spain	1394	3.3	1.2
Canada	1274	3.1	1.1
Italy	1247	3.1	1.1
Australia	1140	2.8	1.0
10 countries	34,391	[a]84.9	30.5
CDG OICs	40,509	100.0	35.6

CDG, country development group; *OICs*, old industrialized countries.
[a]*Sum does not add up because of rounding.*
Source: FAO database. faostat.fao.org. (data of January 16th, 2017).

production volume of this country group; the regional concentration was the second highest of all country development groups.

The main results of the second step of this analysis can be summarized as follows:

- The share in global population and in poultry-meat production is rather imbalanced in all four country development groups.
- The highest imbalance is to be found in the groups of the least-developed and old industrialized countries. While the least-developed countries have a comparatively high share in the global population, they only contribute a small amount to world poultry-meat production. The opposite is the case in the old industrialized countries. They share only about one-seventh of the global population but more than one-third of world poultry-meat production.
- The regional concentration in the four distinguished country development groups differs considerably. It is lowest in the less-developed countries and highest in the NICs.

1.4 THE LEADING POULTRY-MEAT-PRODUCING COMPANIES—AN OVERVIEW

The following paragraph will give an overview on the leading poultry companies on a global and on a continental basis.

The data in Table 1.10 shows that the five leading poultry-meat-producing companies were located in the Americas and in East Asia and Southeast Asia. The Brazilian company JBS (José Batista Sobrinho Sociedade Anónima) ranked in first place with an annual slaughter volume of almost 3.4 billion birds. This includes the purchased companies in the USA and Mexico. In second and third place ranked Tyson and BRF: they slaughtered 2.3 billion and 1.7 billion birds per year, respectively. It is worth mentioning that no European company was listed among the top companies.

In a further step, the leading companies on a continental level will be documented.

In North America, four of the five leading companies were located in the USA and one in Mexico. Mexico is classified as a North American country because of its membership in NAFTA (North American Free Trade Agreement). The size of the companies differed considerably as can be seen

TABLE 1.10 The Five Leading Poultry-Meat-Producing Companies Worldwide (2015)

Company	Country	Birds Slaughtered (million/year)[a]
JBS	Brazil	3380
	USA	
	Mexico	
Tyson	USA	2310
	China	
	Brazil	
	Mexico	
BRF	Brazil	1664
CP Group	Thailand	939
	Indonesia	
	Turkey	
	China	
Wen's Food Group	China	714

[a]*Aggregated slaughter volume of the companies.*
Source: WATTAgNet.

TABLE 1.11 The Five Leading Poultry-Meat-Producing Companies in North America (2015)

Company	Country	Birds Slaughtered (million/year)
Tyson	USA	1862
Pilgrim's[a]	USA	1493
Perdue Farms	USA	654
Koch Foods	USA	624
Industria Bachoco	Mexico	503

[a]*Parent company: JBS Aves (Brazil).*
Source: WATTAgNet.

from the data in Table 1.11. Tyson and Pilgrim's, the two leading companies, slaughtered 1.9 billion and 1.5 billion birds per year, respectively. It has to be considered that Pilgrim's is owned by JBS Aves (Brazil). The production capacity of the three following companies was considerably lower. Industria Bachoco is a vertically integrated poultry company in Mexico, controlling the whole supply chain from breeding, feed production, growing, slaughtering, and further processing. It is also active in swine fattening and pork production.

In South America, three of the five leading companies were located in Brazil, one in Peru, and one in Chile (Table 1.12). In first place ranked BRF Foods, a company which in 2009 originated from a merger of the Brazilian food companies Perdigão and Sadia. San Fernando (Peru) is a food company which, besides poultry meat, also sells turkey meat, eggs, and pork. The Agrosuper Group is the leading Chilean company in producing and marketing of animal proteins (chicken, salmon, and ham). Aurora Alimentos is one of the largest food-producing and processing companies in Brazil. The company is active in the poultry-meat, pork, and dairy market.

In Europe, the five leading poultry-meat-producing companies were located in France, Germany, the Netherlands, Italy, and the United Kingdom (Table 1.13). The size of the five listed companies did not differ very much. They slaughtered between 370 million and 400 million birds annually. All five companies are vertically integrated and active in various fields of food production and processing. Some of the companies are active in several countries; the PHW Group, for example, in Germany, Poland, Bulgaria, and the Netherlands, the LDC Group in France, Poland, and Spain. Both companies are totally or mainly family owned.

Three of the five leading Asian poultry-meat-producing companies were located in China (Table 1.14). If one would, however, aggregate the slaughter volumes of the whole CP Group, it would rank in first place with 939

TABLE 1.12 The Five Leading Poultry-Meat-Producing Companies in South America (2015)

Company	Country	Birds Slaughtered (million/year)
BRF Foods	Brazil	1831
JBS Aves	Brazil	982
San Fernando	Peru	242
Agrosuper Group	Chile	197
Aurora Alimentos	Brazil	188

Source: WATTAgNet.

TABLE 1.13 The Five Leading Poultry-Meat-Producing Companies in Europe (2015)

Company	Country	Birds Slaughtered (million/year)
LDC Group	France	400
PHW Group	Germany	390
Two Sisters	UK	370
Plukon Group	Netherlands	354
Agricola Italiana Alimentare	Italy	350

Sources: WATTAgNet, personal information.

TABLE 1.14 The Five Leading Poultry-Meat-Producing Companies in Asia (2015)

Company	Country	Birds Slaughtered (million/year)
Wen's Food Group	China	714
New Hope Group	China	700
CP Group	Indonesia	600
Doyou Group	China	500
JAPFA	Singapore	312

Sources: WATTAgNet, personal information.

million slaughtered birds per year. The Chinese New Hope Group is the leading agribusiness company in China, producing poultry meat and pork. Besides agricultural products, the company is also active in various other business fields, i.e., chemical products. JAPFA was founded in Indonesia, but is now headquartered in Singapore from where the activities in animal protein production in various countries of Eastern and Southeastern Asia are controlled.

All five leading African poultry-meat-producing companies were located in South Africa (Table 1.15). This is not surprising as this country was leading in African poultry-meat production with a volume of 1.7 million tons in 2014. RCL Foods is active in various fields of food production from primary production to marketing of processed foods. Astral Foods is the leading South African poultry company. It is vertically integrated from hatcheries to broiler growing and processing, and also produces its own feed. The production volumes of the three other companies were much smaller.

The poultry-meat companies in Oceania mainly served regional markets resulting from the peripheral location and their comparatively small population. Therefore the number of slaughtered birds was much lower than in other continents.

The main results of the preceding paragraph can be summarized as follows:

- On a global scale, the largest poultry-meat-producing companies in 2015 were located in North and South America as well as in Eastern and Southeastern Asia.
- Most of the leading companies are vertically integrated and control the whole supply chain in poultry-meat production, some even from breeding, parent stock to marketing of processed products.
- Several of the large companies are multinational enterprises trying to cover several markets with their activities.

TABLE 1.15 The Five Leading Poultry-Meat-Producing Companies in Africa (2015)

Company	Country	Birds Slaughtered (million/year)
RCL foods	South Africa	260
Astral foods	South Africa	229
Country bird holdings	South Africa	74
AFGRI poultry	South Africa	58
Pioneer foods	South Africa	52

Sources: WATTAgNet, personal information.

- Several large companies are active in the whole field of animal protein production, ranging from poultry meat and eggs to pork beef and fish.

1.5 A PROJECTION OF THE DEVELOPMENT OF POULTRY-MEAT PRODUCTION AND CONSUMPTION BETWEEN 2014 AND 2024

In the following paragraph, a projection of the development of poultry-meat production, consumption and per capita consumption will be presented. The projection is based on the OECD-FAO Agricultural Outlook (2015).

Table 1.16 shows the projection of the development of global poultry-meat production on the basis of two development classes. The OECD-FAO projection expects an absolute growth of 24.4 million tons between 2014 and 2024. The classification differs from that in the four country development groups in the first part of this analysis. In the OECD-FAO Agricultural outlook, 40 countries are classified as developed countries. These are the 35 old industrialized countries plus the Russian Federation, Ukraine, Kazakhstan, South Africa, and Croatia. The data shows that production will increase faster in the developing countries than in the developed countries and that according to this classification the developing countries will contribute 60% of the global production volume in 2024.

A projection of the expected development on the basis of continents and selected countries is given in Table 1.17. From the data, one can see that with the exception of Japan, all other countries will show a growth of their production volumes. The highest absolute increase on the continent level will occur in Asia with 7.5 million tons, followed by North America with 5.7 million tons, and Central and South America with 4.8 million tons. These three continents will contribute 74% to the global increase; Asia alone will contribute 30.8%. In Europe, poultry production will grow by only 2.8 million tons, which is less than half the increase of North America.

TABLE 1.16 Projected Development of Global Poultry-Meat Production Between 2014 and 2024 by Development Status (Data in 1000 t)

Development Status	2014	2019	2024	Increase (%)
Developed countries	45,390	49,985	53,575	18.0
Developing countries	64,013	72,179	80,271	25.4
World	109,403[a]	122,164	133,786	22.3

[a]*The difference in the production volume for 214 in comparison to Table 1.1 is due to the original data in the OECD-FAO projection.*
Source: OECD-FAO Agricultural Outlook 2015. OECD Publishing, Paris. http://dx.doi.org/10.1787/agr-outlook-2015-en; own calculations.

TABLE 1.17 Projected Development of Poultry-Meat Production Between 2014 and 2024 on Continent and Country Levels (Data in 1000 t)

Continent/country	2014	2024	Change (1000 t)	Change (%)
Africa	5354	6069	+715	+13.4
South Africa	1536	1847	+311	+20.2
Egypt	782	1051	+269	+34.4
Asia	36,659	46168	+7509	+25.9
China	18,250	23112	+4862	+26.6
India	2651	3521	+870	+32.8
Iran	2051	2380	+329	+16.0
Indonesia	1809	2312	+503	+27.8
Turkey	1809	2172	+363	+20.0
Malaysia	1394	1859	+465	+33.3
Thailand	1366	1739	+373	+27.3
Japan	1508	1496	−12	−0.8
Philippines	1073	1303	+230	+21.4
Korea, Rep.	734	971	+237	+32.3
Saudi Arabia	585	613	+28	+4.8
Europe	19,049	21,816	+2767	+14.5
EU (28)	13,049	14,355	+1306	+10.0
Russian Federation	3973	4864	+891	+22.4
Ukraine	1285	1635	+350	+27.2
North America	24,039	29,771	+5732	+23.8
USA	19,918	24,424	+4506	+22.6
Mexico	2880	3906	+1026	+35.6
Canada	1241	1441	+200	+16.1
Central and South America	21,156	25,974	+4818	+22.8
Brazil	12,914	15,702	+2788	+21.6
Argentina	1979	2557	+578	+29.2
Colombia	1171	1515	+344	+29.4
Chile	689	828	+139	+20.2

(*Continued*)

TABLE 1.17 (Continued)

Continent/country	2014	2024	Change (1000 t)	Change (%)
Oceania Developed	1298	1495	+197	+15.2
Australia	1110	1236	+126	+11.4
New Zealand	188	198	+10	+5.3
World	109,403	133,786	+24,383	+22.3

Sources: OECD-FAO Agricultural Outlook 2015. OECD Publishing, Paris. http://dx.doi.org/10.1787/agr-outlook-2015-en; own calculations; own calculations.

A closer look at the situation on a country level reveals considerable differences. The highest absolute growth is projected for China with 4.9 million tons, followed by the USA (+4.5 million tons), Brazil (+2.8 million tons), and Mexico (+1.0 million tons). These four countries will share 54.1% of the expected increase of global poultry-meat production between 2014 and 2024. The absolute growth in the EU will be much lower than in China, the USA, or Brazil, with only 1.3 million tons which is less than half of the Brazilian growth.

In only five countries the relative growth rates will surpass 30%. These are Mexico (35.6%), Egypt (34.4%), Malaysia (33.3%), India (32.8%), and the Republic of Korea (32.3%). The lowest growth rates are projected for Saudi Arabia (4.8%) and New Zealand (5.3%).

In a next step, the projected development of poultry-meat consumption on continent and country levels between 2014 and 2024 will be analyzed in more detail.

A comparison of the data in Tables 1.17 and 1.18 shows similarities and differences in the dynamics on continent and country levels. This has impacts, as will be demonstrated in a later table, on the balance between production and consumption.

The highest absolute growth of poultry-meat consumption is projected for Asia with 11.7 million tons, followed by North America (+4.2 million tons), and Central and South America (+3.5 million tons). These three continents will share 80.0% of the absolute increase, Asia alone will contribute 48.2%. On a country level, China, the USA, and Brazil are expected to have the highest increases in consumption with 9.9 million tons or 40.4% of the overall growth all together. The term "consumption" in the OECD-FAO Outlook is somewhat misleading, however, as it includes domestic consumption and exports. This has to be considered in particular for the USA and Brazil as the two leading broiler-meat-exporting countries. Japan is the only country with a decreasing consumption. For the EU, a low growth rate is projected. This is mainly due to the already high per capita consumption in 2014.

TABLE 1.18 Projected Development of Poultry-Meat Consumption Between 2014 and 2024 on Continent and Country Levels (Data in 1000 t)

Continent/Country	2014	2024	Change (1000 t)	Change (%)
Africa	6827	9559	+2732	+40.0
South Africa	1858	2401	+543	+29.2
North Africa	2305	3008	+703	+37.0
Sub-Saharan	2664	4150	+1486	+55.8
Asia	39,783	51,517	+11,734	+29.5
China	18,087	23,238	+5151	+28.5
India	2643	3521	+878	+33.2
Iran	1995	2402	+407	+20.4
Indonesia	1810	2318	+508	+28.1
Turkey	1392	1780	+388	+27.9
Malaysia	1401	1863	+462	+33.0
Thailand	600	737	+137	+22.8
Japan	1966	1885	−81	−4.1
Philippines	1189	1518	+329	+27.7
Korea, Rep.	866	1029	+163	+18.8
Saudi Arabia	1448	1715	+276	+18.4
Europe	18,797	20,653	+1856	+9.9
EU (28)	12,536	13,603	+1067	+8.5
Russian Federation	4302	4744	+442	+10.3
Ukraine	1194	1432	+238	+19.9
North America	21150	25,385	+4235	+20.0
USA	16,308	19,492	+3184	+19.5
Mexico	3507	4364	+857	+24.4
Canada	1335	1529	+194	+14.5
Central and South America	22,012	25,519	+3507	+15.9
Brazil	8888	10,407	+1519	+17.1
Argentina	1668	1948	+280	+16.8
Colombia	1224	1604	+380	+31.0

(*Continued*)

TABLE 1.18 (Continued)

Continent/Country	2014	2024	Change (1000 t)	Change (%)
Chile	656	806	+150	+22.9
Oceania developed	1250	1434	+184	+14.7
Australia	1063	1236	+173	+16.3
New Zealand	188	198	+10	+5.3
World	108,587	132,956	+24,369	+22.4

Sources: OECD-FAO Agricultural Outlook 2015. OECD Publishing, Paris. http://dx.doi.org/10.1787/agr-outlook-2015-en; own calculations.

The data in Table 1.19 shows that an increase in the global per capita consumption of poultry meat from 13.19 kg to 14.60 kg or 10.7% is projected for the decade from 2014 to 2024. There are, however, remarkable differences between continents and countries.

The highest average per capita consumption on a continent level in 2024 is projected for North America with 47.80 kg, followed by the developed countries in Oceania with 39.92 kg, Central and South America with 32.77 kg, and Europe with 24.47 kg. It is surprising at first sight that Asia, despite the high production and consumption volumes, only ranks in fifth place with 10.06 kg. Only in Africa, the value is much lower with 4.52 kg, mainly a result of the low consumption in Sub-Saharan countries. The imbalance between total and per capita consumption in Asia is a result of the large population in China, India and Indonesia and the comparatively low per capita consumption in India and Indonesia. The extremely low per capita consumption in India is both due to the high percentage of vegetarians and the low average per capita income.

A more detailed analysis of the data in Table 1.19 and Fig. 1.4 on a country basis reveals the enormous differences in the per capita consumption. For the USA the highest value is projected with 49.3 kg, followed by Malaysia (47.4 kg), Saudi Arabia (45.7 kg), Brazil (42.3 kg), and Australia (40.8 kg). The three countries with the lowest expected per capita consumption are located in Asia: India with only 2.2 kg, Indonesia with 7.3 kg, and Thailand with 9.6 kg. It can be assumed, however, that several countries in Sub-Saharan Africa will have even lower consumption values, but there is no data available on a single-country basis.

The data in Table 1.19 reflect the development status of the countries, the average available income for food purchase, religious taboos, and also the amount of poultry-meat exports. This has to be considered in particular

TABLE 1.19 Projected Development of the Human Consumption of Poultry Meat Between 2014 and 2024 on Continent and Country Levels (Data in kg Ready to Cook Per Person and Year)

Continent/Country	2014	2024	Change (kg)	Change (%)
Africa	4.03	4.52	+0.49	+12.2
South Africa	30.77	37.48	+6.71	+21.8
North Africa	11.65	13.34	+1.69	+14.5
Sub-Saharan	2.57	3.09	+0.52	+20.2
Asia	8.47	10.06	+1.59	+18.8
China	11.42	14.13	+2.71	+23.7
India	1.84	2.20	+0.36	+19.6
Iran	22.37	24.21	+1.84	+8.2
Indonesia	6.30	7.30	+1.00	+15.9
Turkey	16.15	18.86	+2.71	+16.8
Malaysia	40.85	47.44	+6.59	+16.1
Thailand	7.86	9.55	+1.69	+21.5
Japan	13.62	13.41	−0.21	−1.5
Philippines	10.45	11.37	+0.92	+8.8
Korea, Rep.	15.39	17.59	+2.20	+14.3
Saudi Arabia	41.20	41.58	+0.38	+0.9
Europe	22.24	24.47	+2.23	+10.0
EU (28)	21.61	23.14	+1.53	+7.1
Russian Federation	26.57	30.34	+3.77	+14.2
Ukraine	23.37	30.08	+6.71	+28.7
North America	43.35	47.80	+4.45	+10.3
USA	44.49	49.27	+4.78	+10.7
Mexico	24.93	28.03	+3.10	+12.4
Canada	33.06	34.61	+1.55	+4.7
Central and South America	29.95	32.77	+2.82	+9.4
Brazil	38.71	42.33	+3.62	+9.4
Argentina	35.11	37.99	+2.88	+8.2
Colombia	22.02	25.91	+3.89	+17.7

(*Continued*)

TABLE 1.19 (Continued)

Continent/Country	2014	2024	Change (kg)	Change (%)
Chile	32.47	37.02	+4.55	+14.0
Oceania developed	39.04	39.92	+0.88	+2.3
Australia	39.57	40.83	+1.25	+3.2
New Zealand	36.29	35.00	−1.29	−3.6
World	13.19	14.60	+1.41	+10.7

Sources: OECD-FAO Agricultural Outlook 2015. OECD Publishing, Paris. http://dx.doi.org/10.1787/agr-outlook-2015-en; own calculations; own calculations.

TABLE 1.20 Changes in the Marketing of Broilers in the USA Between 1964 and 2014 (Data in %)

Year	Marketing Form		
	Whole Birds	Cut-Up/Parts	Further Processed
1964	80	18	2
1984	31	52	17
1994	12	54	34
2004	10	43	48
2014	11	41	48

Source: US National Chicken Council:www.nationalchickencouncil.org.

for the USA and the countries with a high per capita consumption in South America (Brazil and Argentina).

There is only few data available on how poultry meat is marketed. A long-term overview is available for the USA (Table 1.20). It shows that whole broilers which dominated in 1964 are only of minor importance presently. They were displaced by cut up parts and further processed meat. A similar development can be observed in most EU member countries. In Italy, only 11% of the broilers were marketed as whole bird in 2014, 63% as cut up parts, and 26% in processed form. In most EU countries, fresh poultry meat is favored by consumers. In Germany, 76% of all broiler meat was marketed fresh in 2014. In contrast, almost only whole deep-frozen birds with a ready to cook weight of 800 g to 1000 g are marketed in Saudi Arabia and the United Arab Emirates. Imported cut up parts (leg quarters) are

mainly offered as deep-frozen products in the Russian Federation, China, Japan, and South Africa; fresh products are preferred, however, for domestically produced broilers. In rural areas of the least-developed and also many less-developed countries, broilers and spent hens are consumed fresh as whole birds because of the lack of cold storage facilities.

1.6 PROJECTED SURPLUS AND DEFICIT OF POULTRY MEAT ON CONTINENT AND COUNTRY LEVELS IN 2024

In a final step, the balance between production and consumption will be analyzed at continent and country levels.

From the data in Table 1.21, one can see that Asia and Africa will be the two continents with a high negative balance. Together they will lack about 8.8 million tons of poultry meat to meet the demand of their population. On the other hand, Central and South America as well as North America are the two continents with a surplus of together 9.2 million tons; for Europe, a surplus of 1.2 million tons is projected.

A comparison of the projected production and consumption values reveals a problem which, however, cannot be solved from the available data. There is a calculated surplus of 1.6 million tons if one uses the data for the continents as published in the OECD-FAO statistics, but the expected surplus calculated from the global production and consumption data is only 830,000 t. Quite obviously, the consumption data are too low. Even if one added the consumption of the less-developed countries in Oceania, which can be estimated at 80,000 t, there would still be a considerable difference. It is assumed by the author that the deficit in Asia is higher than documented in the OECD-FAO data as the Central Asian countries seem not to have been considered and also consumption data for West Asia seem to be incomplete.

Despite the data problems, some basic patterns of the future balance between production and consumption of poultry meat are obvious. Brazil and the USA will play a decisive role in broiler meat exports also in the future. The gap between the export volumes between these two countries will, however, become wider and Brazil's export volume will considerably surpass that of the USA. Thailand and Argentina will also remain major poultry-meat exporters, this will also be the case for several EU member countries (Netherlands, Poland, Germany, France, Belgium, United Kingdom).

In 2024, major importing countries will be Saudi Arabia and the United Arab Emirates, Viet Nam, Mexico, and almost all African countries, especially South Africa. Despite their high export volumes, several EU member countries will also import considerable amounts of broiler meat. Although most of the trade will be intra-EU trade, chicken breast fillets and salted cooked broiler meat will be imported from Brazil and Thailand, respectively.

TABLE 1.21 Projected Development of the Balance Between Poultry-Meat Production and Consumption in 2014 on Continent and Country Levels (Data in 1000 t)

Continent/Country	Production	Consumption	Balance
Africa	6069	9558	−3489
South Africa	1847	2401	−554
North Africa	2507	3008	−501
Sub-Saharan	1716	4150	−2434
Asia	46,168	51,517	−5349
China	23,112	23,238	−126
India	3521	3521	0
Iran	2380	2402	−22
Indonesia	2312	2318	−6
Turkey	2172	1780	+392
Malaysia	1859	1863	−4
Thailand	1739	737	−398
Japan	1496	1885	+1002
Philippines	1303	1518	−215
Korea, Rep.	971	1029	−58
Saudi Arabia	613	1715	−1102
Europe	21,816	20,653	+1163
EU (28)	14,355	13,603	+752
Russian Federation	4864	4744	+120
Ukraine	1635	1432	+203
North America	29,771	25,385	+4386
USA	24,424	19,492	+4932
Mexico	3906	4364	−458
Canada	1441	1529	−88
Central and South America	25,974	21,155	+4819
Brazil	15,702	10,407	+5295
Argentina	2557	1948	+609
Colombia	1515	1604	−89

(*Continued*)

TABLE 1.21 (Continued)

Continent/Country	Production	Consumption	Balance
Chile	828	806	+22
Oceania Developed	1495	1434	+61
Australia	1297	1236	+61
New Zealand	198	198	0
World	133,786	132,956	+830

Sources: OECD-FAO Agricultural Outlook 2015. OECD Publishing, Paris. http://dx.doi.org/10.1787/agr-outlook-2015-en; own calculations.

Whether the USA will be able to export broiler meat to the EU will depend on the results of the ongoing TTIP negotiations.

1.7 SUMMARY AND PERSPECTIVES

The preceding analysis demonstrates the worldwide success story of poultry-meat production. The high absolute and relative growth rates are a result of the success in breeding which made strains with an excellent feed conversion and good health available to the industry. The parallel development of housing systems with a high technological standard in climate control, feed, and water supply, as well as vaccination against the main threatening poultry diseases, further contributed to the continuous growth of global poultry-meat production. An important steering factor is also the fact that there are no religious taboos against poultry-meat consumption, and in less-developed countries broilers and spent hens can be consumed as a whole bird because of small carcass size and do not need refrigeration, in contrast to pork and beef meat. This also explains why poultry meat has, apart from egg consumption, become the main protein source for a growing population in many less-developed and threshold countries.

It can be expected that this success story will continue in the next decade. The projected dynamics of production and consumption demonstrate the ever-increasing importance of this sector of animal production as the main protein source for not only the developed but also the less-developed countries. The fact that the food supply of a growing population will have to be realized on a smaller acreage of arable and irrigated land is a convincing argument to increase poultry-meat and egg production because of the favorable feed conversion of broilers and laying hens. The animal welfare and environmental impact discussion in several EU member countries has shown, however, that intense production in large units and the spatial concentration of broiler and egg farms in some regions have led to a growing number of

opponents and a decreasing social acceptance of industrialized animal production. This may in some countries, such as Germany, the Netherlands, and the United Kingdom, result in a stagnating or even decreasing production volume of broiler und turkey meat.

A high risk factor for the future development of the global poultry industry is the introduction and dissemination of the highly pathogenic avian influenza virus. A growing number of documented outbreaks in several Asian and African countries and also in the USA, France, Italy, Germany, the United Kingdom, Poland, and the Netherlands in 2015 and 2016 have shown that the industry will have to live with this threat for years. While wild birds played an important role in the introduction of the virus in the USA, the Netherlands, Germany, Italy, and France, the spatial dissemination has in many cases been a result of a lacking biosecurity on the poultry farms, especially for those adopting farming systems that provide birds with outdoor access. It will be necessary to better educate farmers, in particular in developing and some threshold countries, regarding the improvement of their biosecurity.

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Part I

What is New in Our Understanding of the Association Between Muscle Structure and the Basic Eating Qualities of Cooked Meat?

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Chapter 2

Myogenesis Muscle Growth and Structure

Daniel Clark and Rachel Harding
The Ohio State University, Wooster, OH, United States

2.1 INTRODUCTION

The ultimate goal of the broiler and turkey industries is to produce a high-quality-meat protein source in an economical and environmentally sustainable manner. The three aspects of poultry productivity that should be considered to meet the goals of the industry are meat yield, meat quality, and feed efficiency. Balancing the quality, quantity, and cost of production is a significant challenge, and in many cases altering one factor will affect the others as well. At the core, these three factors are tied together by cellular mechanisms that govern pre- and pos-thatch skeletal muscle growth. This chapter will focus on the fundamental molecular and cellular aspects that govern skeletal muscle growth.

2.2 MUSCLE STRUCTURE

Skeletal muscle is comprised of large cells that are surrounded by an intricate connective tissue network that supports muscle function. The entire muscle is surrounded by a layer of connective tissue known as the epimysium (Fig. 2.1A). The epimysium creates a large supportive structure that aids in forming the shape of the muscle, and provides portals for the entry of nerves and blood vessels. The muscle is made up of individual muscle cells (also known as muscle fibers or myofibers) that are organized into bundles called fasciculi, by a layer of connective tissue called the perimysium. Like the epimysium, the perimysium also forms a collagenous structural support system that is needed for proper muscle function. Another layer of connective tissue known as the endomysium surrounds each individual myofiber. The cell membrane, or sarcolemma, is connected to the endomysium by another membrane called the basal lamina. Mature muscle fibers are multinucleated, with the nuclei residing directly underneath the sarcolemma

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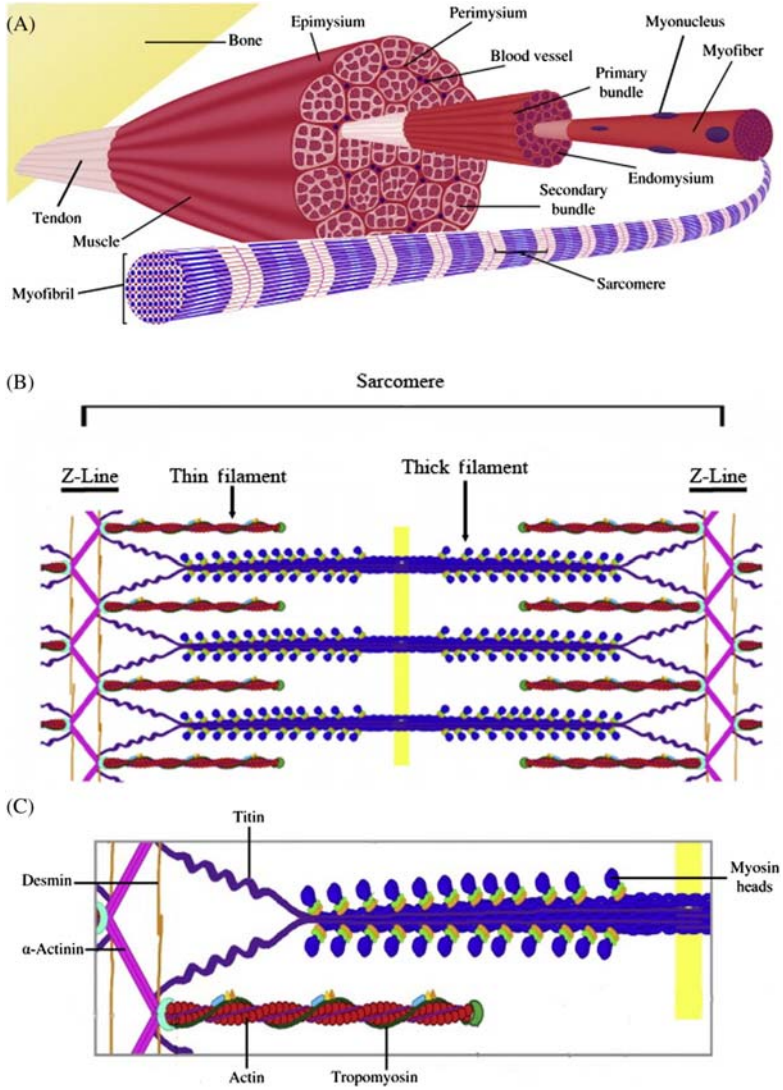


FIGURE 2.1 Illustration of muscle structure and the contractile apparatus. (A) The entire muscle is surrounded by a connective tissue known as the epimysium. Within the muscle, individual myofibers are grouped together into bundles, and the entire bundle is surrounded by the perimysium. Individual myofibers are surrounded by the endomysium. Myofibers are multinucleated and have many myofibrils which are comprised of the contractile proteins that make up the sarcomere. (B) The sarcomere is bordered by Z-lines. The thin filaments are connected to the Z-lines, while thick filaments lie in the center of the sarcomere. (C) The thin filament is comprised of actin, and regulatory proteins such as tropomyosin. The thick filament primarily consists of myosin and is connected to the Z-line by titin. The Z-line contains structural proteins such as desmin and α-actinin. *Adapted from Velleman, S.G., McFarland, D.C., 2014. Chapter 16: Skeletal muscle. In: Scanes, C.G., (Ed.), Sturkie's Avian Physiology, sixth ed. Elsevier Inc., Boston, pp. 379–402.*

(Lawrence and Fowler, 2002; Gerrard and Grant, 2003; Velleman and McFarland, 2014). Variations or disruptions in these connective tissue structures can affect meat quality. For example, the connective tissue structure of the endomysium changes with age and is thought to be one of the primary components of muscle that influences tenderness. Additionally, intramuscular fat depots and capillaries are commonly found within the perimysial space.

2.3 MUSCLE CONTRACTION

Myofibers contain a large number of contractile apparatuses known as myofibrils. Myofibrils are cylindrical microfilamentous organelles with a diameter of approximately 1 μm and typically extend throughout the entire muscle fiber. The smallest fundamental contractile unit of the myofibril is called the sarcomere (Fig. 2.1B), a repeating structure throughout the entire myofibril. Each myofibril is comprised of thick and thin filaments which are the active proteins that drive muscle contraction. Each sarcomere is bordered by structural support proteins, predominantly consisting of α-actinin, that collectively comprise the Z-line (Fig. 2.1C). Thin filaments, primarily composed of actin, but also containing regulatory proteins such as troponin and tropomyosin, are connected to the Z-line. Thick filaments contain myosin, the most abundant myofibrillar protein, and are anchored to the center of the sarcomere by a large elastic protein called titin. During contraction the thick and thin filaments shorten the sarcomere by pulling the Z-lines closer together. Thick and thin filaments are organized in a parallel fashion along the entire length of the myofibril. The repeating patterns gives skeletal muscle a microscopically striated appearance and is the origin of the term "striated muscle" in reference to skeletal muscle (Lawrence and Fowler, 2002; Gerrard and Grant, 2003; Velleman and McFarland, 2014).

Muscle contraction is primarily accomplished by the interaction of myosin and actin through a process known as the crossbridge cycle. Initially, an electrical nerve impulse causes the depolarization of the transverse tubule system, which is an extension of the sarcolemma that aids in the rapid release of calcium from the sarcoplasmic reticulum. After its release, calcium binds to the protein troponin. Troponin and tropomyosin are two regulatory myofibrillar proteins that, under resting conditions, block myosin binding sites located on actin. Once calcium binds troponin, however, a conformational change ensues within the tropomyosin−troponin complex that exposes a myosin binding site on the actin protein. Exposure of the myosin binding site allows myosin to become tightly bound to actin. The formation of the myosin−actin bond is also called a crossbridge, which drives muscle contraction. As myosin binds to actin, ADP and P_i are released from the enzymatic myosin head. This release then causes a conformational change in the neck region of the myosin protein, which slides the thin filament (actin) closer to middle of the sarcomere and brings the Z lines closer together. In

this conformation, ATP binds to the ATPase head of myosin thereby breaking the myosin−actin bond. The ATP molecule is then hydrolyzed into ADP and P_i that again cause the myosin neck to return to its original conformation, and begin the cycle again. When ATP is absent from the system, the myosin head remains attached to actin causing a condition known as rigor mortis (Gerrard and Grant, 2003; Germain and Stanfield, 2005).

2.4 FIBER TYPE

While the fundamental mechanics of the crossbridge cycle are consistent in all myofibers, there are differences in contraction speed between myofiber types. In avian species, it is generally accepted that there are three types of adult skeletal muscle fibers: Type I, slow-twitch oxidative; Type IIa, fast-twitch oxidative/glycolytic; Type IIb, fast-twitch glycolytic. The major difference between fast- and slow-twitch muscle fibers is largely based on the speed of contraction. The speed of contraction is highly related to the myosin ATPase head that is contained within each muscle fiber. In fast-twitch muscle fibers, the ATPase head of myosin hydrolyzes ATP at a much quicker rate than slow-twitch muscle fibers, resulting in more rapid contraction of the fast-twitch fibers.

The differences in contraction speed between fiber types also necessitate different levels of energy availability. For this reason, slow-twitch myofibers and fast-twitch myofibers utilize different types of metabolism. Slow-twitch type I oxidative fibers preferentially produce energy through the oxidative breakdown of glucose or fatty acids (Westerblad et al., 2010). Muscles that are comprised of a high percentage of type I fibers are also highly vascularized, providing a source of circulating oxygen. These muscles also have a high concentration of myoglobin, an oxygen carrying compound that gives these fibers a red color (Velleman and McFarland, 2014) and provides oxygen for oxidative metabolism. There are three main stages of oxidative metabolism: glycolysis, the Kreb's cycle, both of which occur in the cytoplasm, and the electron transport chain, which occurs in the mitochondria (Westerblad et al., 2010). While glycolysis and the Kreb's cycle only net 4 ATP total, the electron transport chain produces an additional 26 ATP for each glucose molecule metabolized, making oxidative metabolism very energy efficient (Matsakas and Patel, 2012). The requirement for mitochondria in oxidative metabolism means that type I oxidative fibers have a high concentration of mitochondria (Velleman and McFarland, 2014).

Fast-twitch type II anaerobic fibers preferentially generate energy through anaerobic metabolism of muscle glycogen or phosphocreatine. When energy stores are low, phosphocreatine can be broken down by creatine kinase to produce creatine and ATP, which can be used as an energy source for contraction (Westerblad et al., 2010). Alternatively, glucose, retrieved from muscle glycogen, proceeds through glycolysis producing a net of 2 ATP

molecules (Westerblad et al., 2010; McDonald and Gladden, 2012). In the absence of oxygen, the end products of glycolysis are hydrogenated, producing lactic acid, which must be shuttled to the liver through the circulatory system to be converted back to glucose. Hence, anaerobic respiration is less efficient than oxidative respiration, as it only nets 2 ATP molecules and lactic acid (McDonald and Gladden, 2012). Type II anaerobic fibers have a low propensity for oxidative metabolism, and therefore have minimal oxidative support through vascularization, and low myoglobin content. The low levels of myoglobin in these muscle types are responsible for the paler "white meat" color compared to the aerobic muscles.

Depending upon the function of the muscle, different muscles contain different proportions of fast- and slow-twitch fibers. For example, some muscles are used for repetitive ongoing tasks, such as maintaining posture or in the case of migratory birds, long distance flight. Muscles used for these tasks are primarily comprised of slow-twitch, fatigue-resistant muscle fibers that utilize efficient oxidative metabolism. Other muscles that are used for powerful, quick movement are primarily comprised of white, fast-twitch muscles that can quickly utilize muscle glycogen stores, but fatigue quickly. Muscle fibers also exhibit plasticity, shifting from one fiber type to another depending on the needs of the muscle (Gerrard and Grant, 2003). Modern commercial broilers have been selected for energy efficiency and greater growth. This selection has resulted in modern birds that are nearly flightless with a *Pectoralis major* muscle comprised entirely of anaerobic muscle fibers (Smith and Fletcher, 1988; Branciari et al., 2009). On the other hand, the Leghorn genotype of chickens have had minimal selection pressure for accelerated growth, and when raised in conventional, indoor facilities (Branciari et al., 2009) have a small percentage (0.5%) of aerobic fibers in the *P. major* muscle. Yet, due to the plasticity of skeletal muscle fibers, the percentage of aerobic fibers has been shown to increase (3.8%) when Leghorn chickens are reared in organic systems, which is likely due to an increase in activity (Branciari et al., 2009). Dependent upon the use, different muscles can also vary in their respective compositions of aerobic or anaerobic muscle types. For instance, the semimembranosus leg muscle of modern commercial Ross broilers is primarily used for posture and support and as a result is comprised of approximately 37.1% anaerobic, white fibers, and 60.9% red fibers (Branciari et al., 2009).

2.5 EMBRYONIC ORIGIN OF MUSCLE

The complete embryonic phase of growth is a complex, extensive process and is beyond the scope of this text; however, to comprehend the mechanisms that mediate posthatch muscle growth and meat quality, it is first imperative to obtain a basic understanding of skeletal muscle development. To facilitate this understanding, this section will cover the fundamental

principles that govern embryonic myogenesis (for an in-depth review, see Gilbert, 2000; Wolpert et al., 2011). Early in development, skeletal muscle arises from the mesoderm layer of the embryo. After the notochord develops, pairs of somites form from mesodermal cells through a process called somitogenesis and develop along the neural tube. Somite formation begins at the rostral (near the head) end of the embryo in a very organized pattern (Gerrard and Grant, 2003). As somites form, the neural tube, notochord, and lateral plate mesoderm secrete different growth factors such as bone morphogenic protein 4 (BMP-4), sonic hedgehog (Shh) and Wnt (Wolpert et al., 2011). The release of these factors, also known as morphogens, forms signaling gradients across the somites which specify somite patterning. These growth factor signaling gradients will allow for the formation of two distinct cell populations: the dermamyotome and sclerotome (Fig. 2.2). High levels of Shh, released from the neural tube and notochord, specify the somatic cells that are closest to the neural tube as sclerotomal cells, which will give rise to the vertebral column and ribs. Low levels of Shh coupled with high levels of Wnt specify the cells in the dorsal region of the somite as the dermomyotome. The dermomyotome then forms two layers called the dermatome and the myotome (Wolpert et al., 2011). The dermatome will give rise to the dermal tissue, while the myotome will give rise to a population of proliferative cells termed myoblasts, which are responsible for forming skeletal muscle (Fig. 2.3). The myoblast cells near the developing limbs will migrate along the limb buds to form the skeletal muscle of the limbs. The remaining myoblasts will migrate along the axial skeleton to form the skeletal trunk muscles, such as the *P. major* and *Pectoralis minor* (Wolpert et al., 2011).

As myoblasts begin to migrate they commit to a myogenic lineage, shifting focus from proliferation to myogenic differentiation. This determination process is accomplished by a family of muscle regulatory transcription factors (MRFs): myogenic determination factor 1 (MyoD), myogenic regulatory factor 4 (MRF4), myogenic factor 5 (Myf5), and myogenin. Myogenic determination factor 1, MRF4, and Myf5 are responsible for initiating the

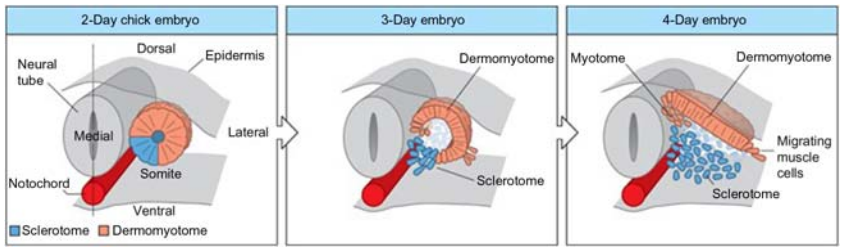


FIGURE 2.2 Somatic cell fate in a chick embryo. The quadrant closest to the notochord (ventral medial) will give rise to the sclerotomal cells that form the vertebral column. The remainder of the cell will contribute to the dermomyotome. The dermomyotome will give rise to dermal cells and myoblasts. *Courtesy of Wolpert, L., Tickle, C., Lawrence, P., Meyerowitz, E., Robertson, E., Smith, J., et al., 2011. Principles of Development, fourth ed. Oxford University Press, Oxford.*

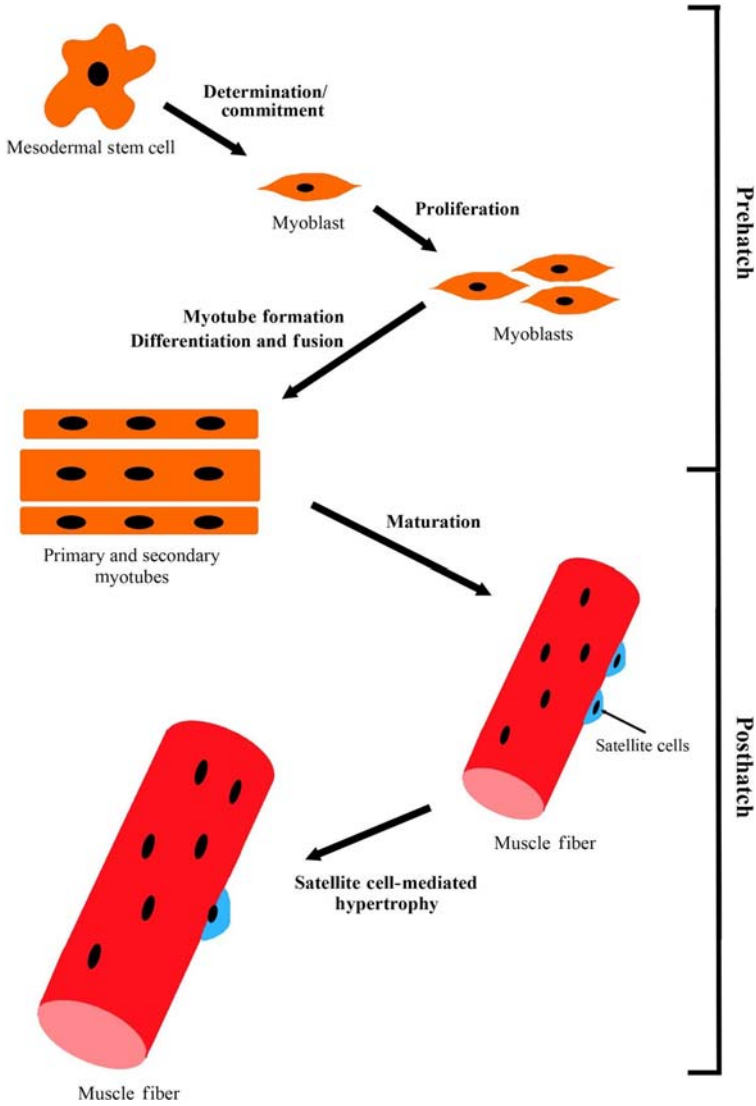


FIGURE 2.3 An overview of muscle formation and growth. Myoblasts originate from an embryonic mesodermal stem cell. During embryogenesis, myoblasts, commit to a myogenic lineage, proliferate then differentiate into primary and secondary myotubes. After hatching, muscle fibers increase in size through a process called hypertrophy. Hypertrophy increases the size of a myofiber by increasing the protein production capacity of that myofiber through donation of nuclei by satellite cells.

myogenic determination program. Committed myoblasts maintain their proliferative capacity for a period of time (Fig. 2.3); however, once proliferation ceases, myoblasts will begin to terminally differentiate (Wolpert et al., 2011). The differentiation process takes place in two waves. The first wave

of myoblasts forms primary myotubes, which establish the scaffolding for the formation of additional myotubes. The second wave of myotube formation is complete at the time of hatching (Fig. 2.3). Immature myotubes can be histologically characterized by centrally located nuclei. As the muscle fiber matures and the contractile apparatus (myofibrils) develop, the nuclei are pushed to the periphery of the muscle fiber (Gerrard and Grant, 2003). It is important to note that at the time of hatch, embryonic myoblasts have completely withdrawn from the cell cycle, and myotube formation is complete. The number of muscle fibers that are present will not change throughout the bird's lifetime.

2.6 POSTHATCH MUSCLE GROWTH

Once differentiated, myofibers are not capable of proliferation. All posthatch muscle growth is accomplished by enlargement of individual muscle fibers through a process called hypertrophy. The rate of hypertrophic growth is directly related to the relative rates of protein synthesis and protein degradation, which change as animals age. For example, in young chicks that are undergoing a rapid period of growth, a high rate of protein synthesis and relatively low rate of protein degradation ultimately allow for a rapid increase in the accumulation and deposition of protein. As the chicken ages and reaches a mature size, the rates of protein synthesis and degradation reach an equilibrium, as a result protein accumulation is relatively stable (Schreurs, 2000).

As muscle mass increases, protein synthesis capabilities must also increase. Therefore, hypertrophic growth is accomplished by increasing the protein synthesis capabilities of existing myofibers by addition of myonuclei (Fig. 2.3). The source of these additional myonuclei is an adult myogenic stem cell population called satellite cells (Smith, 1963; Hawke and Garry, 2001). First described by Mauro (1961), satellite cells are located between the basal lamina and sarcolemma of the muscle fiber. In poultry, satellite cells are most active the first week posthatch, mediating the rapid muscle accretion occurring during this time (Halevy et al., 2000). After this time adult satellite cells maintain a minimally active state, as necessary for ongoing growth and normal repairs. In order to mediate muscle growth or repairs, satellite cells must enter the cell cycle and proliferate to produce additional cells that migrate to the appropriate area of muscle growth, align, and fuse with existing myofibers. This fusion allows the integration of the satellite cell nuclei into the existing myofiber, thereby increasing protein production capabilities and allowing muscle mass accretion to occur (Moss and LeBlond, 1971; Campion, 1984). This same process is utilized by satellite cells to repair muscle injuries accrued due to injury or disease (Chargé and Rudnicki, 2004; Collins et al., 2005; Yin et al., 2013).

Satellite cells are an adult stem cell population, and therefore have been found to display a wide range of heterogeneity. Subpopulations of satellite cells

have been identified that have a number of variable characteristics including proliferation rates (McFarland et al., 1995; Schultz, 1996; McFarland et al., 1997; Kuang et al., 2007; Rossi et al., 2010; Manzano et al., 2011), responsiveness to growth factors and signaling molecules (McFarland et al., 1997; McFarland et al., 2003), myogenic potential (Lagord et al., 1998; Yada et al., 2006; Rossi et al., 2010; Powell et al., 2014; Harding et al., 2015), and expression of myogenic regulatory factors (Lagord et al., 1998; Manzano et al., 2011). Some evidence exists that faster-growing populations tend toward myogenic differentiation, while the slower-growing populations are more likely involved in self-renewal of the satellite cell population (Schultz, 1996; Kuang et al., 2007; Rossi et al., 2010). Satellite cells from different fiber types in poultry have also been found to have intrinsic differences (McFarland et al., 1995, 1997; Harding et al., 2015). For example, it has been demonstrated that chicken *P. major* satellite cells are more responsive to increasing serum levels than B. femoris satellite cells (McFarland et al., 1997). Additionally, *P. major* satellite cells are more sensitive to temperature change in vitro than satellite cells from the B. femoris muscle (Harding et al., 2015). Growth selection in poultry has also been found to cause intrinsic changes in satellite cells, as satellite cells isolated from a body weight selected turkey line are more sensitive to temperature change than satellite cells from a random-bred control turkey line (Clark et al., 2016). These examples demonstrate that the satellite cells exist in a heterogeneous population with a wide range of characteristics that may vary between one subpopulation and the next.

2.7 MYOGENIC REGULATORY FACTORS IN MUSCLE GROWTH

Much of satellite cell identity, function, and heterogeneity can be defined by the expression of specific transcription factors. The expression of Paired box 7 (Pax7) in satellite cells begins during embryonic development and continues into the adult satellite cell. The expression of Pax7 helps determine satellite cell identity, and promotes proliferation (Seale et al., 2000; Oustanina et al., 2004; Kuang et al., 2006; Relaix et al., 2006; Kuang et al., 2007; Kuang and Rudnicki, 2008). To date, Pax7 is the only known gene expressed in all satellite cell subpopulations, making it an excellent satellite cell marker (Chargé and Rudnicki, 2004).

All satellite cells express Pax7, but as a stem cell, they are also capable of self-renewal and differentiation to alternative lineages (Asakura et al., 2001; Shefer et al., 2004). Satellite cell commitment to a myogenic lineage begins with Myf5 expression (Pownall et al., 2002; Zammit et al., 2006; Kuang et al., 2007; Kuang and Rudnicki, 2008). The expression of Pax7 persists in satellite cells expressing Myf5, creating a $Pax7^{+}/Myf5^{+}$ satellite cell population. As the satellite cells continue through the differentiation process, they begin to express MyoD, which is an essential promoter of satellite cell

proliferation (Yablonka-Reuveni and Rivera, 1994; Halvey et al., 2004; Olguin and Olwin, 2004; Zammit et al., 2004). Following proliferation, MyoD expression decreases to allow for the creation of $Pax7^+$ quiescent satellite cells, or to allow for myogenin expression, leading to differentiation (Halvey et al., 2004; Olguin and Olwin, 2004; Zammit et al., 2004). Myogenin is a transcription factor required for the expression of genes that are essential for skeletal muscle differentiation (Brunetti and Goldfine, 1990; Osses and Brandan, 2002). Another marker of myogenic determination is myogenic regulatory factor 4 (MRF4). During development, MRF4 is initially expressed with MyoD, but is downregulated until it is primarily expressed in the late embryonic stage, when myotube formation is detectable (Hintenberger et al., 1991; Zhang et al., 1995). Expression of MRF4 persists in the adult myofiber and has been found to be necessary for the downregulation of myogenin following differentiation (Hintenberger et al., 1991; Zhang et al., 1995).

2.8 REGULATION OF MUSCLE GROWTH

There are many different biological components that mediate skeletal muscle growth. Hormones and growth factors are endogenously produced and have numerous effects on many different systems throughout the body. Hormones and growth factors can directly stimulate a cellular response by three different mechanisms. Autocrine signaling occurs when a cell secretes a factor which then acts on the same cell to elicit a response. Paracrine signaling is a cell to cell signaling method in which factors that are released from one cell act upon another nearby cell. The final form of cell to cell communication is endocrine signaling. Endocrine signaling occurs when growth factors or hormones are produced by various tissues throughout the body and secreted into circulation. These endocrine factors will then be delivered to the target tissue to elicit a response. Hormones are considered to be any type of chemical messenger secreted by a variety of tissues (Gerrard and Grant, 2003). Growth factors are occasionally referred to as hormone-like polypeptides that are generally produced locally and act in an autocrine or paracrine manner (Gerrard and Grant, 2003). Some growth factors, such as insulin-like growth factor (IGF) family members, are also produced in the liver and therefore can act in an endocrine, as well as an autocrine or paracrine manner.

Numerous growth factor families have been identified that mediate skeletal muscle growth such as IGF, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), and transforming growth factor beta (TGFβ). A more comprehensive review of these growth factor families and the mechanisms by which they regulate skeletal muscle growth has previously been published (McFarland, 1999). The following is a brief overview of three different critical growth factor families, IGF, FGF, and TGFβ, which govern skeletal muscle growth.

The IGF family of growth factors contains two polypeptide ligands, IGF1 and IGF2. Both factors share a similar structure and function to insulin. As with insulin, both of these factors not only promote skeletal muscle growth, but also have metabolic effects. Both IGF1 and IGF2 stimulate the type I IGF receptor (IGF1R) to mediate growth effects (Duclos et al., 1991; Sun et al., 1992). Activated IGF1R initiates two downstream signaling cascades: PI3K/AKT and MAPK/ERK, which stimulate protein synthesis. (Janeczko and Etlinger, 1984; Duclos et al., 1993; McFarland et al., 1994). Unlike many other growth factors, IGF1 and IGF2 stimulate both proliferation and differentiation of myoblasts and satellite cells (McFarland et al., 1993). Exogenous IGF1 and IGF2 also increase glucose transport in both chicken and turkey embryonic myoblasts and satellite cells (McFarland et al., 1994), which is needed for increased cellular metabolism. Insulin-like growth factor function is also mediated by IGF binding proteins (IGFBP). Insulin-like growth factor binding proteins are secreted by many cell types and have high affinities for IGF1 and IGF2 (Clemmons, 1997). Differential affinities for IGF1 and IGF2 and temporal expression patterns allow IGFBP to precisely alter signaling at different points throughout development (Clemmons, 1997).

Fibroblast growth factors are a large family of growth factors that are heavily associated with heparin sulfate proteoglycans of the extracellular matrix (ECM). Fibroblast growth factors have a major role in embryonic limb development and myoblast proliferation. Satellite cells also require FGF for proliferation, to the extent that satellite cells are unable to proliferate in vitro in FGF-free media (McFarland et al., 1991, 1993). Evidence also suggests that FGF reduces myoblast and satellite cell differentiation (McFarland et al., 1997, 2000). It has been hypothesized that a reduction in FGF receptors is required for the differentiation of satellite cells and myoblasts (Itoh et al., 1996).

Another family of growth factors, the TGFβ family, inhibits skeletal muscle development and growth. Transforming growth factor β family members are widespread during both embryonic and posthatch growth. One member of the TGFβ family, myostatin, is specifically produced within skeletal muscle. Defects that render the myostatin gene nonfunctional are responsible for the double-muscled phenotype found in Belgian blue and piedmontese breeds of cattle (McPherron and Lee, 1997; Grobet et al., 1997). Myostatin, TGFβ1 and TGFβ2 have been shown to inhibit both embryonic myoblast and satellite cell proliferation and differentiation (Yun et al., 1997; McFarland et al., 2006). Myostatin is also hypothesized to be involved in skeletal muscle degeneration, as it is elevated during myofiber breakdown, repressing satellite cell activation (Guernec et al., 2003). For regeneration to occur, myostatin expression must be reduced to allow satellite cells to repair damaged muscle (Guernec et al., 2003).

In addition to the many polypeptide growth factors, hormones also mediate growth. Growth hormone is produced by the pituitary gland and is released in the circulatory system as an endocrine factor (Scanes, 2014). In

mammals, growth hormone administration results in an increase in skeletal muscle mass and a reduction in fat accumulation (Scanes, 2009). In avian species, however, the biological function of growth hormone is less clear. In young chicks and poults, administration of exogenous growth hormone has minimal effect on growth (Cogburn et al., 1989; Proudman et al., 1994). Alternately, in chickens, the removal of the pituitary gland, which reduces circulating growth hormone, depresses growth by over 50% (King and Scanes, 1986). In turkeys, removal of the pituitary gland has less of an effect on growth, reducing average daily gain by 30% in one study (Proudman et al., 1994), while other studies report no effect on growth (Proudman and Opel, 1990). For a more in-depth review of the current understanding of the physiological function of growth hormone see Harvey (2013) and Scanes (2014).

2.9 EXTRACELLULAR MATRIX REGULATION OF MUSCLE GROWTH

The ECM is composed of all of the molecules secreted by the cell and immobilized in the surrounding environment. In skeletal muscle, the ECM is primarily comprised of collagens, non-collagenous glycoproteins, and proteoglycans. Historically thought to be a static support for the cell, the ECM has since been found to be a dynamic and interactive framework involved in cell support, migration, and signaling. Signals are transmitted through the ECM and directed into the cell through a number of cell-surface receptors and signaling molecules including proteoglycans. Proteoglycans are composed of a core protein with covalently attached glycosaminoglycan (GAG) chains. The GAG chains are made up of disaccharide polymers that are highly sulfated. When attached to proteoglycans, the different types of GAG chains allow for classification of proteoglycans as heparan sulfate proteoglycans (HSPGs), chondroitin sulfate proteoglycans, or dermatan sulfate proteoglycans (Velleman, 2012; Brandan and Gutiérrez, 2013). The sulfated nature of the GAG chains gives them a strong negative charge, allowing for interaction with polar molecules such as water or growth factors (Velleman, 2012). The number, type, and placement of the GAG chains vary widely between proteoglycans, and can interact with either the extracellular or intracellular environment. The placement and type of attached GAG chains partially dictate the function of proteoglycans, either contributing to cell signaling, or mediating cellular adhesion and cytoskeletal organization, thereby providing structural integrity to the cell (Brandan and Gutiérrez, 2013). For a more in-depth review of proteoglycans see Brandan and Gutiérrez (2013).

Collagen, the most abundant extracellular macromolecule, is found in the connective tissue layers of muscle (perimysium, epimysium, and endomysium) where it provides a supportive framework and prevents overstretching of muscle. There are numerous different types of collagen throughout the

body; however, the most prevalent isoforms found in skeletal muscle are types I and III (Bailey et al., 1979; McCormick, 1999). Collagen is comprised of three polypeptide chains that form a right-handed triple helix. The most common motif in the polypeptide sequences are repeats of glycine-X-Y, where X and Y are commonly lysine or proline (McCormick, 1999; Velleman and McFarland, 2014). To become mature, collagen must go through a series of posttranslational modifications including glycosylation of lysine residues and hydroxylation of other lysine or proline residues. Collagen fibers are comprised of mature single collagen fibrils that are secreted into the ECM by cells. The collagen fibrils align in a quarter-stagger array, such that each fibril overlaps approximately one-quarter of its length. This array is then stabilized by a series of cross-links (Velleman and McFarland, 2014). There are two types of crosslinks, the divalent or ketoamine crosslink and the trivalent or hydroxypyridinium (HP) crosslink. The ketoamine crosslink is temporary, reversible, and is replaced by the mature nonreversible HP crosslink. The numbers of HP crosslinks increase as an animal ages, and along with a shift to more type I collagen, is highly associated with tougher, lower-quality meat (McCormick, 1999).

The formation of collagen crosslinks is mediated by decorin, a small leucine-rich proteoglycan containing either chondroitin sulfate or dermatan sulfate GAG chains. Decorin is comprised of 10 leucine-rich repeats in the central domain and contains at least one high-affinity fibrillar collagen binding site (Weber et al., 1996). In vitro experiments suggest that decorin directly interacts with the fibrillar collagen binding site to facilitate collagen alignment and crosslinking (Vogel et al., 1984; Vogel and Trotter, 1987; Scott, 1988; Danielson et al., 1997). This is supported in vivo, as mice lacking functional decorin have irregular collagen fibrils (Danielson et al., 1997). Decorin has also been associated with skeletal muscle disorders and myopathies. For instance, the Low Score Normal (LSN) condition is a skeletal muscle disorder that causes chickens to have a reduced ability to right themselves after being placed on their back (Velleman et al., 1996). The LSN disorder is associated with very high decorin levels in the *P. major* muscle and an approximately 200% increase in collagen HP crosslinking (Velleman et al., 1996). One of the muscle myopathies more recently associated with decorin, Wooden Breast, occurs in the *P. major* muscle of broilers. The Wooden Breast myopathy is often found in fast-growing, heavy broilers, and like the LSN condition, is associated with very high levels of decorin and increased collagen. In Wooden-Breast-affected muscles, collagen is tightly packed in a parallel fashion, suggesting an increase in HP crosslinks (Fig. 2.4; Velleman and Clark, 2015).

In addition to its role mediating collagen crosslinking, decorin has several functions in the muscle regulating growth factor signaling. The core protein of decorin can directly bind and sequester TGFβ and myostatin, both strong inhibitors of muscle growth. Decorin can also directly bind to connective

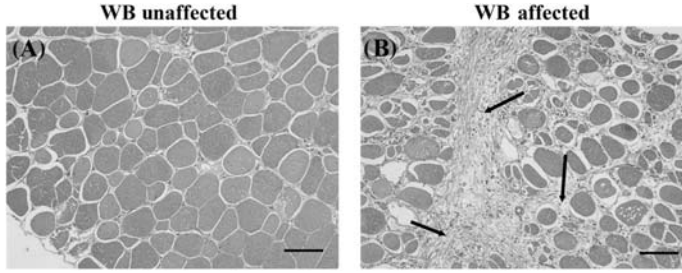


FIGURE 2.4 Photomicrographs of transverse sections of the *Pectoralis major* from broilers that are unaffected (A) or affected by the Wooden Breast myopathy (B). *Arrows* highlight perimysial connective tissue accumulation. Scale bar = 100 μm. *Modified from Velleman, S.G., Clark, D.L., 2015. Histopathologic and myogenic gene expression changes associated with Wooden Breast in broiler breast muscles. Avian. Dis. 59, 410–418.*

tissue growth factor, thereby inhibiting its fibrotic effects. In addition, decorin can interact with growth factor receptors, such as IGF1R and epidermal growth factor receptor (EGFR), to either initiate down-stream signaling cascades or inhibit signaling, depending upon the cell type. For a more in-depth review of the interactions between decorin and growth factor signaling pathways, see Kinsella et al. (2004).

2.10 CELL SURFACE PROTEOGLYCANS: MEDIATORS OF ECM SIGNALING

There are a variety of proteoglycans that are excreted into the ECM and regulate signal transduction; however, there are also cell surface proteoglycans with a similar role. A number of cell surface proteoglycans act as co-receptors for essential growth factors including FGF2, which is a strong promoter of satellite cell proliferation, and inhibitor of differentiation. Two of the most thoroughly studied cell surface proteoglycans are the syndecan and glypican families.

There are four known syndecan family members, syndecan-1, syndecan-2, syndecan-3, and syndecan-4, all of which are found in skeletal muscle (Velleman, 2012). All four syndecan family members are transmembrane HSPGs with an extracellular domain, a transmembrane domain, and a cytoplasmic domain. In addition to the structural similarities, all four syndecans have similar, but non-overlapping functions. Syndecan-1 is highly expressed during embryonic myoblast proliferation, acting as an FGF2 signal regulator (Velleman 2012; Brandan and Gutiérrez, 2013). Syndecan-2 is also primarily expressed during proliferation of myoblasts and appears to be involved in TGFβ signaling (Velleman, 2012; Brandan and Gutiérrez, 2013). Syndecan-3 appears to be necessary for myogenic satellite cell survival and proliferation, as knockdown of syndecan-3 results in a cell cycle halt and an increase in cell death (Cornelison et al., 2004; Velleman, 2012; Brandan and Gutiérrez, 2013).

Syndecan-4, the most widely studied syndecan, is primarily expressed in satellite cells, where it is essential for proliferation and migration (Cornelison et al., 2004; Brandan and Gutiérrez, 2013).

Syndecan-4, in conjunction with integrins, forms areas of strong cell attachment to the surrounding substrate called focal adhesions (Dovas et al., 2006; Woods and Couchman, 1994). Focal adhesions are frequently attached to stress fibers, which are actinomyosin contractile fibers that anchor the cell to the surrounding ECM. Both focal adhesions and stress fibers are essential for cell migration, providing a strong anchor point from which the cell can pull or push itself along. When syndecan-4 is activated, the cytoplasmic domain oligomerizes, interacts with phosphatidylinositol-4-5-bisphosphate (PIP2) and activates protein kinase Cα (PKCα) (Oh et al., 1997, 1998; Dovas et al., 2006; Shin et al., 2013). The activation of PKCα is essential for the downstream activation of RhoA, a small GTPases necessary for both focal adhesion and stress fiber formation (Dovas et al., 2006; Shin et al., 2013). As syndecan-4 is the first step in a signaling cascade that ultimately leads to RhoA activation, it is essential to satellite cell migration.

Another HSPG known to be essential to satellite cell function is glypican-1. While syndecan-3 and syndecan-4 are mainly involved in the proliferation and survival of satellite cells, glypican-1 appears to primarily regulate satellite cell differentiation (Brandan and Gutiérrez, 2013). Glypican-1 is an extracellular protein anchored to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor. Glypian-1 regulates differentiation by regulating FGF2 signaling to satellite cells. The glypican-1 protein is anchored in areas of concentrated sphingolipids and cholesterol, called lipid rafts, in the plasma membrane (Gutiérrez and Brandan, 2010; Brandan and Gutiérrez, 2013; Gutiérrez et al., 2014). Glypican-1 regulates FGF2 signaling by binding and sequestering FGF2 along the lipid rafts, away from the tyrosine kinase receptors present in other regions of the plasma membrane. Additional research has shown that glypican-1 can also be cleaved from its GPI anchor, allowing shedding into the ECM, where it also binds and sequesters FGF2 (Velleman et al., 2013). In either form, glypican-1 prevents FGF2 from binding to tyrosine kinase receptors on the cell surface, thereby preventing FGF2-mediated satellite cell proliferation and allowing myogenic differentiation to occur (Gutiérrez and Brandan, 2010; Velleman et al., 2013). Glypican-1 expression is likely also upstream of myogenin, a gene essential to proper myogenic differentiation, as knockdown of glypican-1 results in decreased myogenin expression (Gutiérrez and Brandan, 2010).

2.11 THE EFFECT OF GROWTH SELECTION ON MYOGENESIS AND MUSCLE GROWTH

The poultry industry has met consumer demand for a lean-meat, high-quality protein source by placing a significant emphasis on selection for energy-efficient,

fast-growing, high-yielding broilers and turkeys. This has resulted in broilers that reach processing by 6 weeks of age with a breast muscle that is nearly 900% larger than broilers produced in the 1950s (Collins et al., 2014). The increase in muscle growth and breast yield has resulted in changes to the morphology and molecular properties of the *P. major* muscle that negatively impact meat quality (Wilson et al., 1990; Velleman et al., 2003). As discussed previously in this chapter, the number of muscle fibers are fixed at the time of hatching, therefore continued selection for posthatch muscle growth has led to increased muscle fiber size rather than increased fiber number (Dransfield and Sosnick, 1999). Increasing fiber size reduces the amount of endomysial and perimysial space within the muscle (Velleman et al., 2003), leading to a reduction in space for vascularization. Without sufficient capillary support, essential nutrients are unable to be delivered to the muscle and metabolic waste products such as heat and lactic acid will build up in the muscle (Velleman, 2015). It has been previously hypothesized that these challenges are, at least in part, responsible for many myopathic conditions and muscle defects that are increasing in both incidence and severity (Velleman, 2015). Examples of these defects found in the breast muscle include: deep pectoral myopathy, focal myopathy, white striping, pale, soft and exudative (PSE) meat, and wooden breast. The metabolic nature of the *P. major* has been hypothesized to contribute to the increased incidence of myopathies and will be discussed to a greater extent in chapter 3.

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Chapter 3

Muscle Metabolism and Meat Quality Abnormalities

Massimiliano Petracci[1], Francesca Soglia[1] and Cécile Berri[2]
[1]*University of Bologna, Bologna, Italy,* [2]*URA, INRA, Nouzilly, France*

3.1 INTRODUCTION

Selection criteria for broiler chickens has been adapted through the decades according to the evolution of the market demand for chicken meat and increased population growth. In the past, poultry-meat production was linked to a model primarily based on self-consumption and direct selling of live or slaughtered animals, but in a few decades there has been a quick transition to an industrialization of poultry production and marketing through modern distribution channels and catering of a very wide range of products ranging from whole carcasses to ready meals (Fletcher, 2004). Within this context, during recent decades the increasing demand for further-processed products, coupled with a preference for breast meat in Western countries (Table 3.1), has shifted selection toward birds with high breast development (Petracci et al., 2015).

The reasons for the increasing demand for chicken breast meat are attributed to its healthy nutritional profile, and the sensory properties that make breast meat very flexible for any type of home-cooking style as well as for manufacturing processed products. Additionally, the mild flavor and the high tenderness of breast meat allow a wide range of desired flavor profiles and textures to be imparted to processed meat products that meet market needs by targeting different groups of consumers. Finally, breast meat is very suitable for quick and easy home-cooking, which is very important in modern societies where people tend to spend increasingly less time on preparation of meals at home (Petracci et al., 2013a).

As a consequence, the poultry industry has been pushed to increase breast yield and to produce heavier birds for further processing (Kuttappan et al., 2016). However, it also has to deal with meat quality issues that were not serious or significant when most poultry was sold as whole birds (Barbut et al., 2008).

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TABLE 3.1 Progress of Broiler Performance and Evolution of Market Segments and Forms of Chicken Meat in the USA

Year		Broiler Performance					Market Segments		Market Forms		
	Market Age (days)[a]	Market Weight (kg)[a]	Average Daily Gain (g/d)[a]	Feed to Meat Gain (g/g)[a]	Mortality (%)[a]	Breast Yield (%)[b]	Retail Grocery (%)[a]	Food-Service (%)[a]	Whole (%)[a]	Cut-up Parts (%)[a]	Further Processed (%)[a]
1945	84	1.37	16.36	4	10	–	–	–	–	–	–
1955	70	1.39	19.89	3	7	13.5	–	–	–	–	–
1965	63	1.58	25.06	2.4	6	–	–	–	78	19	3
1975	56	1.71	30.46	2.1	5	–	75	25	61	32	7
1985	49	1.90	38.79	2	5	–	71	29	29	53	18
1995	47	2.12	45.07	1.95	5	15.8	58	42	10	53	36
2005	48	2.44	50.75	1.95	4	18.6	55	45	11	43	46
2015	48	2.83	58.97	1.87	4.8	21.5	55	45	11	40	49

[a] *NCC (2017).*
[b] *Petracci et al. (2015).*

Consequently, the substantial genetic progress made in the past 50 years has resulted in a reduction in the growing time by about one-half to obtain a market weight bird (Havenstein et al., 2003; Zuihdof et al., 2014). Moreover, in the past 10 years breast-meat yield of the same hybrid has been increased by 3%, which currently exceeds one-fifth of the weight of the bird (Petracci et al., 2015) (Table 3.1).

However, breast muscles, due to their anaerobic metabolism, are more prone to develop metabolic-related defects or myopathies affecting the nutritional value, and the sensorial and technological quality of the resulting breast meat. In the breast muscles of modern broilers, muscle fiber and fiber bundles occupy areas originally maintained by the endomysial and perymisial connective tissue layers, respectively. Thus, spacing between endomysial and perymisial connective tissue is often reduced and this may limit space available for capillaries and hence reduce the amount of lactic acid removed from the muscle. Inadequate elimination of metabolic intermediate products can compromise fiber functionality and result in homeostatic dysregulation. In particular, an increase in the concentration of calcium may activate some proteolytic and lipolytic enzymes leading to membrane dysfunction and increased plasma concentration of enzymes such as creatine kinase and lactate dehydrogenase (Mitchell, 1999; Sandercock and Mitchell, 2003; Petracci and Cavani, 2012; Velleman, 2015).

As a consequence, selection toward increased growth rate and breast yield over the past 30 years has largely affected muscle structure and metabolic status, and increased the incidence of several breast-meat abnormalities such as deep pectoral myopathy (DPM), pale, soft, and exudative (PSE) syndrome, and more recently muscle-growth-related abnormalities such as white-striping, wooden breast, and spaghetti meat (Table 3.2).

This chapter discusses the most recent studies that describe the adverse impacts of the more frequent breast-meat abnormalities and provides details on the implications for the poultry industry.

3.2 POSTMORTEM MUSCLE METABOLISM-RELATED ABNORMALITIES

The quality of poultry meat is a complex concept that involves various criteria and depends on a large number of factors influencing variation, related both to the animal and to its environment (genetics, nutrition, rearing practices, and slaughter conditions). Such factors are able to influence meat composition and structure and also all the biochemical and physical processes that occur in postmortem muscles.

TABLE 3.2 Relationships Between the Evolution of Marketing Forms, Selection Criteria of Chicken Broilers, and Appearance of Breast Meat Abnormalities

Year	Main Form of Commercialization	Main Selection Criteria	Meat Quality Abnormalities
1940	Live bird	Live productivity	–
1960	Carcass	Live productivity Carcass yield	–
1980	Cuts	Live productivity Carcass yield Part yield	Deep pectoral myopathy (DPM)
2000	Cuts and processed products	Live productivity Carcass yield Part yield Meat yield	Deep pectoral myopathy (DPM) PSE-like
2010	Cuts and processed products	Live productivity Carcass yield Part yield Meat yield	Deep pectoral myopathy (DPM) PSE-like White striping Wooden breast Spaghetti meat

Source: Adapted from Petracci M., Mudalal S., Soglia F., Cavani C., 2015. Meat quality in fast-growing broiler chickens. Worlds Poult. Sci. J., 71, 363–374.

Of these factors, the fall in pH that occurs at the death of the animal appears to be a key element for poultry meat quality. It affects the functional properties of muscle proteins and thus a large number of meat quality attributes such as color, water-holding capacity, texture, and shelf life. The postmortem pH fall is characterized by its initial rate, evaluated through measurement of the muscle pH 15 min postmortem (pH_{15}), and its amplitude, corresponding to the pH value reached 24 h postmortem (referred as ultimate pH or pH_u). The initial rate of pH (pH_{15}) is essentially determined by a bird's physical activity before death (such as wing flapping on the shackle line), while ultimate pH mainly depends on the quantity of glycogen available in the muscle at death, also called glycolytic potential (Debut et al., 2003; Berri et al., 2005). Increased wing flapping at slaughter results in decreased pH_{15} (Chabault et al., 2012) while increase in muscle glycogen content results in decreased pH_u (Le Bihan-Duval et al., 2008). Variations in muscle are mainly observed in slow-growing chicken lines (such as those used for free-range production) because of their high levels of reactivity at slaughter (Debut et al., 2005; Chabault et al., 2012). On the other hand,

variations in quality attributes in standard broilers are mainly associated with variations in the pH_u of meat and therefore glycogen storage at slaughter (Le Bihan-Duval et al., 2008). Changes in the kinetics of pH decrease mainly affect the white glycolytic pectoral muscles because of their high levels of glycogen and greater glycolytic activity compared to red thigh muscles (Rémignon et al., 1995; Debut et al., 2003). Normal pH_u values of broiler breast meat are around 5.8 (Duclos et al., 2007; Petracci et al., 2009): the more pH_u deviates from this value, the more defects occur (Fig. 3.1). Meat with high pH_u values (>6.1) exhibits physicochemical properties that result in DFD (dark, firm, and dry) syndrome, while meat with low pH_u (<5.7) is associated with acid meat, often referred to as PSE-like syndrome in broiler meat (Barbut, 1997; Woelfel et al., 2002; Petracci et al., 2004). PSE-like meat is also characterized by high cooking loss and poor marinade uptake (Barbut et al., 2005), meaning that low pH_u values result in economic losses.

Metabolic changes occur in muscle during animal's growth. For example, the glycogen content in breast muscle decreases with age (Table 3.3), which probably explains the higher pH_u observed in older broilers (Baéza et al., 2012). The glycolytic activity (i.e., the production of energy from glycogen through an anaerobic process) in chicken breast muscle increases with age (Rémignon et al., 1995), which could explain why the availability of glycogen in muscle decreases with age. It has also been shown that glycogen storage in breast muscle decreases with the increase in muscle fiber size for a given age (Berri et al., 2007). The increase in breast muscle weight and yield in chickens is mainly achieved through muscle fiber hypertrophy. At the same time, a slight shift toward higher pH_u values (5.9 vs 5.8 on average) has been observed (Debut et al., 2003; Berri et al., 2005, 2008; Baéza et al.,

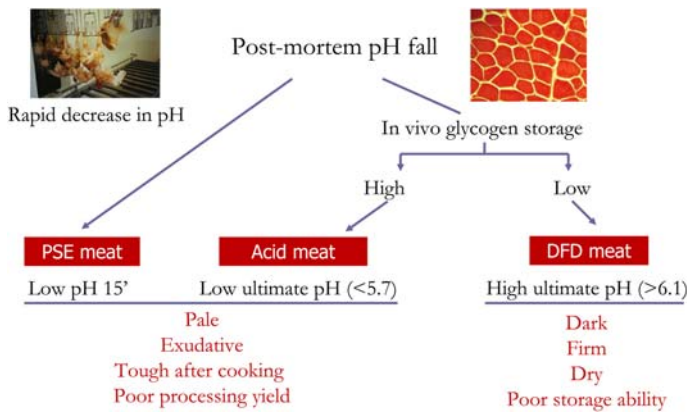


FIGURE 3.1 Variations in muscle pH onset during postmortem time and occurrence of quality defects.

TABLE 3.3 Evolution of the Breast Muscle Glycolytic Potential (Expressed in μm of Equivalent Lactate) With Age in Three Chicken Genotypes Differing in Growth Rate and Age at Slaughter

	Fast-Growing	Medium-Growing	Slow-Growing
2 weeks	95.6[a]		
4 weeks	79.4[b]		
6 weeks	73.4[b]	88.8[a]	106.1[a]
7 weeks		84.1[a]	
8 weeks		69.2[b]	103.2[a]
12 weeks			83.3[b]
Age effect	$P < 0.001$	$P < 0.01$	$P < 0.01$

[a,b]*Means with different letters in the same column are significantly different* ($P \leq 0.05$).

2012), probably corresponding to lower glycogen content in breast muscle in genotypes selected for high growth and muscle yield. Moreover, there are significant positive and negative correlations between cross-sectional area of muscle fiber and ultimate pH or glycolytic potential in chicken pectoral muscle, more generally affecting the quality of breast meat (Berri et al., 2007). This may explain why all breeding practices (selection, nutrition, increasing age at slaughter) that result in increased muscle fiber size (and therefore pH_u) results in a shift toward lower values for lightness, drip and cooking loss, and toughness in broiler breast meat (Baéza et al., 2012; Berri et al., 2007, 2008; Le Bihan-Duval et al., 2008).

The relationships between body composition, muscle metabolism, and breast-meat quality have been specifically evaluated in experimental and commercial models of selection. The first experimental model consisted of a chicken line specifically selected for increased breast-meat yield (+21%) and reduced carcass adiposity (−25%) compared to its unselected control (Le Bihan-Duval et al., 1999; Berri et al., 2001). In this case, body weight at slaughter was similar between the two lines, which allowed the assessment of only the effect of improvement in body composition on muscle and meat quality traits. Such selection resulted in breast muscle that contained less glycogen (−10%) and iron (−22%), resulting in higher pH_u, and lower lightness, and drip loss during storage. The specific relationship between carcass adiposity and breast-meat quality was recently highlighted through the comparison of two chicken lines divergently selected for abdominal fatness (fat and lean lines). These two lines exhibited similar growth rates, but very different abdominal fat percentage (3.9% vs 1.4% in the fat and lean lines,

respectively). The lean line was also characterized by a 10% higher breast-meat yield than the fat line. The lean line had less glycogen (−16%) in the breast muscle, leading to the production of meat with a higher pH_u, and lower lightness and drip loss than the fat line (Sibut et al., 2008). This evolution was confirmed in a commercial model consisting of a line selected for high growth and breast-meat yield and an unselected control line slaughtered at the same age (Berri et al., 2001). In this case, the selected line was 2.2 times heavier and had a breast-meat yield that was 63% higher than the control line. The main consequences for *Pectoralis major* muscle were decreased glycolytic potential (−32%) and iron content (−27%), corresponding to higher pH_u and lower redness of the meat, respectively. A shift to lower oxidative activity was also highlighted in the muscle of selected birds through the measurement of HAD (β-hydroxyacyl coenzyme A dehydrogenase) activity that participates in the β-oxidation of fatty acids in the mitochondria.

Together these studies show that selection programs to improve growth performance and body composition (i.e., increased breast-meat yield and reduced carcass adiposity) have had significant effects on muscle metabolism. The shift toward more glycolytic activity observed would probably explain the lower availability of glycogen and iron in muscle as well as the changes observed in terms of postmortem metabolism and meat quality. Such an evolution has been until a certain point mainly favorable because it improves muscle protein functionality, especially its water-holding capacity during storage and further processing. However, given that progress in terms of growth continues, it is important to ascertain the extent to which the reduction in energy and pigment content may have affected muscle function and health during an animal's life, especially in the actual context where the occurrence of degenerative defects, such as the white-striping, wooden breast, spaghetti meat, and DPM, increases rapidly in broiler strains highly selected for growth and muscle yield.

Several genetic studies have demonstrated the strong genetic determinism of breast quality traits related to postmortem pH fall, and therefore the possibility of improving them by selection. This has been demonstrated in both standard broilers and slow-growing chicken lines that are used for free-range production (Le Bihan-Duval et al., 2001, 2008; Chabault et al., 2012) (Table 3.4). Whatever the population and the breast-meat trait, heritability levels are very high, mostly between 0.3 and 0.6. They are the same as (or above) those for production traits, which suggests the possibility of selecting for such traits at least as effectively as for growth or body composition. All the studies highlighted strong genetic correlations between pH_u and several quality parameters such as lightness, drip loss, and texture after cooking. Because of its high heritability and influence on other quality parameters, breast-meat pH_u can therefore be considered a good candidate for selection

TABLE 3.4 Estimates of Heritability of Breast-Meat Quality and Body Traits in Various Broiler Populations

	Experimental Line	Commercial Standard Line	Commercial Slow-Growing Line
Reference	Le Bihan-Duval et al. (2001)	Le Bihan-Duval et al. (2008)	Chabault et al. (2012)
Body weight/age at slaughter	1659 g/6 weeks	2141 g/6 weeks	2678 g/12 weeks
Heritability			
pH_{15min}	0.49 ± 0.01	0.30 ± 0.05	0.34 ± 0.08
pH_u	0.35 ± 0.03	0.34 ± 0.06	0.48 ± 0.08
Lightness (L*)	0.50 ± 0.03	0.35 ± 0.05	0.44 ± 0.10
Redness (a*)	0.57 ± 0.02	0.25 ± 0.05	0.39 ± 0.10
Yellowness (b*)	0.55 ± 0.04	0.31 ± 0.06	0.30 ± 0.11
Drip loss	0.39 ± 0.04	0.26 ± 0.04	0.30 ± 0.12
Cooking loss	ND	0.35 ± 0.05	ND
Warner–Bratzler shear force	ND	0.34 ± 0.05	0.22 ± 0.08
Body weight at slaughter	0.35 ± 0.02	0.49 ± 0.06	0.46 ± 0.09
Breast meat yield	0.55 ± 0.01	0.30 ± 0.04	0.46 ± 0.12
Abdominal fat percentage	0.62 ± 0.03	0.48 ± 0.06	0.53 ± 0.14

ND, not determined

to improve breast-meat quality in chickens. A divergent selection was initiated at INRA (France) in 2009 from a standard grand-parental commercial female line. The selection program was very efficient since the average difference in pH_u between the two lines was almost 0.5 after six generations of selection (Alnahhas et al., 2014). Physiologically, such divergence of pH_u corresponded to a difference in glycogen of almost 20%. Interestingly, it also affected the pH_u of the *Sartorius* thigh muscle, a 0.3 difference observed after six generations of selection, suggesting a global rather than a specific effect on muscle energy metabolism. Moreover, a genetic correlation of about 0.5 was found between the pH_u of the *P. major* and *Sartorius* muscles (Alnahhas et al., 2015). As expected, the consequences for breast-meat quality were very high, especially for color, water-holding capacity, and texture of cooked meat. More originally, this model also highlighted another defect

of acid meat, i.e., increased susceptibility to oxidation during storage. After six generations of selection, about 60% of the meat could be qualified as "acid" ($pH < 5.7$) or DFD-like ($pH > 6.1$) in the pH_u- and pH_u+ lines, respectively (Alnahhas et al., 2015). Another important result was that selection on the pH_u of breast meat did not affect the growth of broilers or the adiposity of the carcass. However, breast and thigh meat yields were higher in the pH_u+ line than in the pH_u- line (+0.6 and +0.4 points for breast and thigh, respectively). This is consistent with the significant genetic relationships previously shown between breast muscle yield and muscle glycogen storage ($rg = -0.60$) and pH_u ($rg = 0.84$) (Le Bihan-Duval et al., 2008). Such results are very important because they demonstrate the possibility of improving both broiler breast-meat yield and functional properties by selection. However, a higher incidence of white-striping was observed in the pH_u+ compared to the pH_u- line as well as a positive genetic association between white-striping severity and muscle pH_u (Alnahhas et al., 2016) suggesting a possible relationship between the ability of muscle to store energy as a carbohydrate and its likelihood of developing white-striping. Recently, nuclear magnetic resonance (NMR) metabolomics profiling of the breast muscle and serum isolated from these two chicken lines provided information on the metabolic pathways used by each line to produce energy in the muscle. Because of their high ability to store glycogen in muscle, animals in the pH_u- line used carbohydrate as the main source of energy, whereas those in the pH_u+ line used energy produced from amino acid catabolism and lipid oxidation, leading to an adaptive response to oxidative stress (Beauclercq et al., 2016). These first observations made on the pH_u+ and pH_u- lines are extremely interesting since they underline the likely role of a decrease in energy status in several metabolic and cellular processes that are also involved in the occurence of emerging defects such as white-striping, wooden breast, and spaghetti meat (Mutryn et al., 2015; Abasht et al., 2016; Baldi et al., 2017).

3.3 MUSCLE-GROWTH-RELATED ABNORMALITIES

3.3.1 Deep Pectoral Myopathy

Deep pectoral myopathy (DPM), also known as Oregon disease or green muscle disease was firstly described in 1968 as a "degenerative myopathy" affecting turkeys and it was subsequently studied at Oregon State University. Even though this condition was firstly observed to occur in spent turkey and chicken breeds, it was also observed in fast-growing birds about 30 years ago (Siller, 1985). Nowadays, although there are no reported cases in slow- and medium-growing chickens, the occurrence of this myopathy still represents a relevant quality issue for the poultry plants processing fast-growing broilers. In 2006, the incidence of DPM was estimated to attain 1% in heavy broilers weighing more than 3 kg (Bianchi et al., 2006). In addition, Lien

et al. (2011) reported that the incidence of spontaneous cases of DPM ranged from 3% to 17% and, occurring more often in roosters rather than in hens, seemed to positively correlate with increased growth dynamics.

In Poland, a 0.02%−1.9% incidence of DPM was assessed on thousands birds slaughtered in commercial conditions (Kijowski et al., 2014) with 67% of the muscles being bilaterally affected by this myopathy. More recently, in an extensive survey performed in Iran, the occurrence of DPM was found to reach about 0.03% (Pajohi-alamoti et al., 2016).

Even if no health implications are associated with the occurrence of DPM, aesthetically it is definitely undesirable. As a consequence, while all the abnormal fillets should be cut off, the rest of the carcass is still fit for human consumption. However, the trimming operations that are usually adopted to eliminate the affected area within each pectoral muscle leads to meat downgrading thus resulting in economic losses for the poultry industry. In the recent years, since an increasing incidence of DPM is associated with the appearance of damaged areas also within the *P. major* (congestions in the sternum area, hemorrhages) muscles, an overall increased economic impact resulting from trimming and discarding was observed. In addition, the presence of DPM also causes significant commercial complaints when whole carcasses are sold to small cut-up or processors units or butcher shops (Petracci and Cavani, 2012).

Previous studies demonstrated that selection for increased muscle size led to altered vascular structure and thus blood circulation within the deep pectoral muscle. Within this context, if compared to their light-type counterpart, the exercise-stimulated *Pectoralis minor* muscle of heavy-type strains exhibited a one-fifth increased pressure within the fascial compartment (Martindale et al., 1979). These findings revealed that the DPM myopathy is originated by an ischemic process brought about by an increased internal pressure within the pectoral muscle leading to a loss of blood supply following the occlusion of cranial and pectoral arteries and resulting in a necrotic lesion. In detail, aside from the type of poultry, the skeletal muscle increases in weight by about 20% during activity. As a result, because of its anatomical compartmentalization, *P. minor* muscle becomes first strangulated and later ischemic (especially in case of heavy-type broilers) (Siller, 1985). However, the reasons underlying the limited compartment available in high-breast yield birds are currently unknown.

The incidence of DPM is certainly related to the tremendous development of the pectoral muscles in modern commercial hybrids, but it has also been favored by the relatively limited activity during the growing period. Indeed, *Pectoralis minor* muscles are confined in an inelastic compartment that is insufficiently enlarged if these muscles are poorly exercised by the birds (Siller, 1985). As evidenced by previous studies performed under organic conditions, this lack of movement in fast-growing chickens is mainly due to a genetic predisposing factor rather than to the intensive farming

environment (Branciari et al., 2014). Recently, Lien et al. (2012) found that DPM begins to appear at approximately 26 and 36 days of age in male and female broilers, respectively. Increased bird activity (flock nervousness, flightiness, struggle, and wing flapping) induced by factors such as feed or water outages, lighting intensity and programs, human activity as well as excessive noises around chicken houses are looked at as a trigger for the development of DPM in broilers. As selection for breast-meat yield is continuing and broilers are growing to heavier body weights, the occurrence of DPM will likely continue to increase (Lien et al., 2012). Some authors suggested that creatine kinase levels could be used as tools in genetic selection programs to mark the susceptibility of muscle for green muscle disease (Kijowski et al., 2014). However, Lien et al. (2011) indicated that basal creatine kinase does not appear to be related to DPM susceptibility and that a reduced creatine kinase response to early encouraged wing flapping may indicate susceptibility.

3.3.2 Emerging Muscle Abnormalities

In recent years, a new group of muscular abnormalities mainly affecting high-breast yield and fast-growing broilers have appeared (Petracci et al., 2015; Kuttappan et al., 2016) (Fig. 3.2). They include: (1) the white-striping

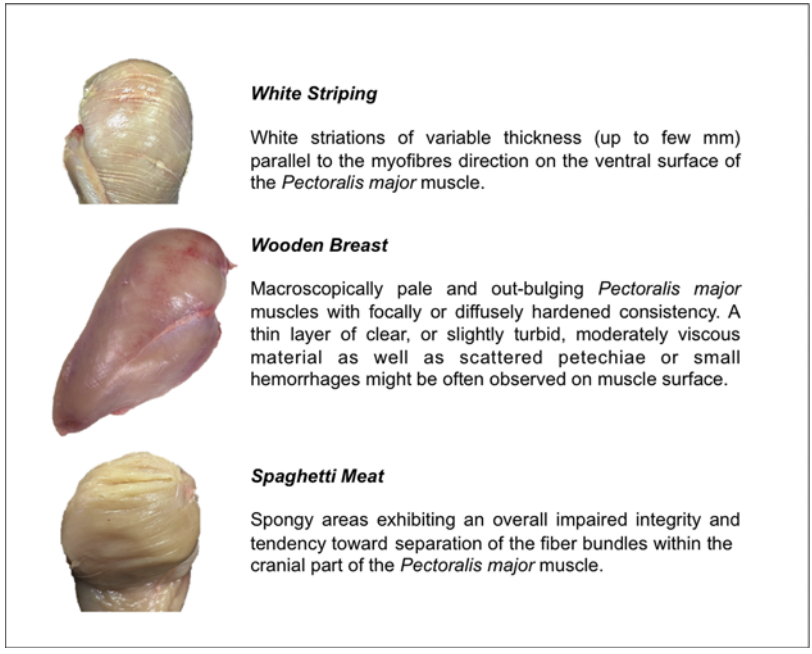


FIGURE 3.2 Macroscopic appearance of *Pectoralis major* muscles affected by growth-related emerging muscle abnormalities.

defect exhibiting white striations parallel to the muscle fibers on the ventral surface of breast fillets (Kuttappan et al., 2009); (2) the woody breast condition (often associated with the white-striping defect) in which the affected muscles are visually hard, bulging out and exhibiting pale color (Sihvo et al., 2014); and (3) the poor cohesion or "spaghetti meat" abnormality described as the tendency toward separation of the muscle fiber bundles composing the *P.major* muscle itself (Sirri et al., 2016; Baldi et al., 2017).

3.3.2.1 Histological Features and Possible Causative Mechanisms

According to previous studies (Table 3.5), irrespective of the type of abnormality, the histological features of the skeletal muscles affected by muscular myopathies were found to overlap. Indeed, similar histological alterations including profound degenerative myopathic changes (focal and diffuse necrosis) resulting in atrophic fibers which typically loose their cross-striations were found in association with occasional regenerative processes, as proved by the presence of thin fibers exhibiting faint cross-striations and nuclear rowing. In addition, variability in fiber cross-sectional area, proliferation of loose connective tissue, and fat deposition (fibrosis and lipidosis) were observed within the endomysial and perimysial spaces in which also interstitial edema and inflammatory cells infiltrates were identified. Similar lesions were also found to affect other muscles composing legs and back (*anterior latissimus dorsi*) of the carcass (Zimerman et al., 2012; Kuttappan et al., 2013a).

Recent studies demonstrated that giant, hyaline (hypercontracted) and necrotic fibers might be observed also within the *P. major* muscles showing no macroscopic lesions ascribable either to the white-striping or to the wooden breast defects (Mazzoni et al., 2015; Soglia et al., 2016a). The presence of necrotic fibers has previously been reported in fast-growing broilers (Soike and Bergmann, 1998; MacRae et al., 2006) and turkeys (Sosnicki et al., 1991) since the 1990s. Similarly, although typically observed within the *P. major* muscles affected by muscle myopathies, giant fibers are commonly found in the histological cross-sections of fast-growing hybrids (Sihvo et al., 2014, 2017; Mazzoni et al., 2015). Indeed, previous studies demonstrated that selection for increased growth rate and breast yield led to a relevant increase in muscle fiber diameter by inducing hypertrophy and hyperplasia of the existing fibers (Rémignon et al., 1995; Burke and Henry, 1997; Scheuermann et al., 2004; Berri et al., 2007) which, in turn, is frequently associated with a higher incidence of giant fibers (Dransfield and Sosnicki, 1999; Le Bihan-Duval et al., 2003; Miraglia et al., 2006). Thus, the presence of myofibers of different diameter and giant fibers alone should not

TABLE 3.5 Main Effects of Emerging Abnormalities on Histological Features, Quality, and Technological Traits of Broiler Breast Meat

Emerging Muscle Abnormalities	
Histological Features	
Profound degenerative myopathic changes	Kuttappan et al. (2009, 2013a), Sihvo et al. (2014, 2017), De Brot et al. (2016), Mazzoni et al. (2015), Soglia et al. (2016a), Clark and Velleman (2016), Kawasaki et al. (2016), Radaelli et al. (2016), Baldi et al., 2017
Presence of abnormal fibers exhibiting rounded profile	
Occasional regenerative processes	
Variability in fibers' cross-sectional areas	
Proliferation of loose connective tissue (fibrosis)	
Fat deposition (lipidosis)	
Interstitial edema	
Inflammatory cells infiltrates	
Vasculitis	
Quality Traits	
Impaired nutritional value	Petracci et al. (2014), Mudalal et al. (2014)
Higher amount of moisture, fat and collagen	Kuttappan et al. (2012), Soglia et al. (2016a,b), Zambonelli et al. (2016), Baldi et al., 2017
Reduced protein content and decreased protein solubility and functionality	Petracci et al. (2014), Mudalal et al. (2014, 2015), Bowker and Zhuang (2016)
Increased ion levels and altered sodium and calcium homeostasis	Sandercock and Mitchell (2004), Wallace and McNally (2009), Soglia et al. (2016a), Zambonelli et al. (2016)
Higher amount of linoleic acid	Soglia et al. (2016b)
Reduced Δ5 and Δ6 desaturase activity	Jordan et al. (1964), Soglia et al. (2016b)
Lower content of anserine, carnosine and creatine	Sundekilde et al. (2017)
Increased yellowness and pale color	Kuttappan et al. (2009), Soglia et al. (2016a)
Increased ultimate pH	Petracci et al. (2013b), Mudalal et al. (2015), Trocino et al. (2015), Abasht et al. (2016), Soglia et al. (2016a), Zambonelli et al. (2016), Tasoniero et al. (2016), Baldi et al. (2017), Baldi et al., 2017

(*Continued*)

TABLE 3.5 (Continued)

Emerging Muscle Abnormalities	
Reduced and altered profile of muscular contractile and sarcoplasmic proteins	Mudalal et al. (2014), Soglia et al. (2016a); Bowker and Zhuang, 2016, Baldi et al., 2017
Reduced oxidative stability	Soglia et al. (2016b)
Technological Properties	
Reduced water holding and water binding capacities: impaired marinate uptake, cooking loss and yield	Mudalal et al. (2014), Petracci et al. (2014), Mudalal et al. (2015), Trocino et al. (2015), Tijare et al. (2016), Soglia et al. (2016a,b), Tasoniero et al. (2016)
Increased proportion and mobility of extra-myofibrillar water fraction	Soglia et al. (2016b), Baldi et al., 2017
Elevated compression and MORSE forces (raw meat)	Petracci et al. (2013b), Mudalal et al. (2015), Soglia et al. (2016a), Chatterjee et al. (2016), Tasoniero et al. (2016)
Increased TPA hardness and chewiness (cooked meat)	

be considered as a criterion to distinguish between the unaffected cases and muscles affected by muscular abnormalities. Mild-to-severe structural abnormalities were found within the *P. major* muscles of fast-growing broiler hybrids (Mazzoni et al., 2015). Thus, not only the fillets noticeably affected by muscle abnormalities, but also the macroscopically unaffected cases exhibited structural modifications such as mild myodegeneration in association with the presence of abnormal fibers (Soike and Bergmann, 1998; Mahon, 1999; Mazzoni et al., 2015; Sihvo et al., 2017). It is thus clear that a complex etiology is associated with the occurrence of these recent muscular myopathies and abnormalities affecting the fast-growing genotypes.

Although several histological investigations have been performed to describe the main microscopic processes taking place within the muscles affected by abnormalities, the histological traits are not specific enough to distinguish the different muscle myopathies from each other. In early studies performed by Kuttappan et al. (2013a) and Ferreira et al. (2014), profound degenerative myopathic lesions leading to myofiber degeneration and occasional regeneration, floccular/vacuolar degeneration, mild mineralization and inflammation of the interstitial spaces with edema as well as lymphocyte and macrophage infiltrations were associated with the occurrence of white-striping abnormality. In addition, the white-striped muscles simultaneously exhibited polyphasic changes involving muscle-fiber fragmentation and

phagocytosis, even if proliferation of connective tissue was not evident in broilers slaughtered at earlier ages. The hematologic and serologic profiles assessed on white-striped meat suggested that the occurrence of this muscular abnormality could not be associated either to infectious, inflammatory mechanisms or to stress conditions. However, the increased levels of creatine kinase in association with serologic enzyme profiles observed in white-striped meat are similar to those observed for other degenerative conditions such as stress and muscular dystrophies resulting in muscle damage (Kuttappan et al., 2013b).

Several studies investigated the histological traits of the wooden breast cases (Sihvo et al., 2014; Mazzoni et al., 2015; Soglia et al., 2016a; Sihvo et al., 2017). It was not possible to identify specific histological patterns for distinguishing between the white-striping and the wooden breast cases. Indeed, polyphasic myodegeneration, rounded fibers, and nuclear internalization often associated with lymphocytic infiltrations and occasional regenerative processes were observed within the woody *P. major* muscles. In addition, proliferation and diffuse thickening of the endomysial and perimysial connective tissue associated with granulation tissue and increased deposition of loose connective tissue (fibrosis) and fat deposition were found to affect the wooden breast fillets (Sihvo et al., 2014; Mazzoni et al., 2015; Soglia et al., 2016a). In addition, in agreement with Kuttappan et al. (2013a), Sihvo et al. (2014), vasculitis and irregular perivascular infiltrations of lymphocytes (sometimes disrupting the vascular wall) affect also the endomysial and perymisial connective tissue of the wooden breast cases (Mazzoni et al., 2015). Since the occurrence of the white-striping and the wooden breast abnormality is frequently combined within the same *P. major* muscle, Soglia et al. (2016a) investigated whether the simultaneous occurrence of thesedefects exert an effect on the histological traits of skeletal muscle. A complete reorganization of the skeletal muscle structure involving the replacement of damaged and necrotic muscle fibers (accompanied by interstitial inflammatory infiltrates) with proliferation of connective tissue was found. In addition, as a result of the degenerative and contextual regenerative processes taking place, muscle fibers exhibited rounded profiles, variable cross-sectional areas, and myofiber numbers (Soglia et al., 2016a). However, both morphological characteristics and histopathological lesions observed within the affected cases were not uniform through the *P. major* muscle and gradually decrease moving from the external surface toward the inner section (about 1-cm-deep) of the *P. major* muscles leading to remarkable modification of the muscular architecture (Clark and Velleman, 2016; Soglia et al., 2016a). Within this context, since these muscle myopathies and abnormalities led to similar histological features, a common underlying mechanism responsible for their occurrence might be hypothesized. Thus, different studies have been performed in order to identify the underlying mechanisms for these alterations. According to the schematic diagram reported in Fig. 3.3, it can be

assumed that in the case of white-striping abnormality, the increased growth rate (especially of breast muscles) achieved through the selection of fast-growing hybrids might have resulted in unsustainable pressure on muscle metabolism, overstretching and/or ischemia of the muscular tissue leading to muscle damage and inducing reparative responses. Additionally, the increased free radical production and accumulation of intracellular calcium may alter the integrity of the muscle fiber membrane and promote protein degradation through activation of proteases and lipases. This process might lead to degenerative and necrotic processes that overtake the regenerative capacity of muscle thus leading to lipidosis and fibrosis (Kuttappan et al., 2013a).

A similar underlying mechanism was hypothesized to be the basis for the wooden breast abnormality. In detail, a higher amount of glycolytic fibers with increased cross-sectional area and reduced capillary-to-fiber ratio was observed within the *P. major* muscles of fast-growing broilers (Hoving-Bolink et al., 2000). As a result, both muscular oxygenation (hypoxic condition) and metabolic waste products displacement might be impaired leading

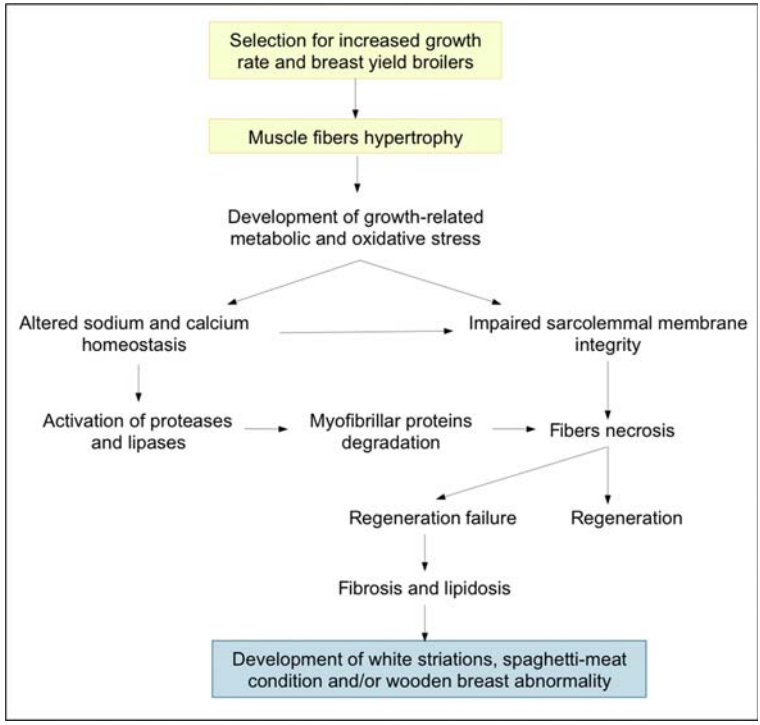


FIGURE 3.3 Schematic diagram displaying the underlying mechanisms hypothesized at the basis of the occurrence of the growth-related emerging muscle abnormalities.

to an excessive accumulation of reactive oxygen species responsible for oxidative stress and involved in initiating inflammatory processes within the muscle tissue (Mutryn et al., 2015; Zambonelli et al., 2016). Then, complex biological reactions and regenerative processes aimed at contrasting muscle inflammation and limiting necrosis and apoptosis take place. Once more, altered calcium homeostasis and glucose metabolism originate when the degenerative processes resulting from inflammation overtake the regenerative capacity of the muscle tissue. Although to date it is reasonably difficult to define which features of the disease appear primarily and secondarily, a differential expression of several genes is associated with the occurrence of muscular abnormalities. In detail, several genes not only exerting a relevant role in inflammatory processes, extracellular-matrix synthesis (with particular reference to proteoglycans) and muscle development, but also involved in polysaccharide metabolic pathways, glucose metabolism, and calcium signaling pathway were up- or downregulated within the abnormal *P. major* muscles (Mutryn et al., 2015; Zambonelli et al., 2016). Intriguingly, the increased expression of hypoxia-related genes (Mutryn et al., 2015; Zambonelli et al., 2016) in association with the presence of tubular structures resulting from neovascularization accompanying the myodegenerative processes in affected muscles corroborate the central role exerted by hypoxia in promoting muscle myopathies and abnormalities.

3.3.2.2 Implications on Meat Quality Features

As expected, the occurrence of muscle myopathies and abnormalities severely affected quality traits and technological properties of meat with the alterations being more pronounced when more than one abnormality coexists within the same muscle. The main implications of muscular abnormalities on meat quality features are summarized in Table 3.5.

Within this context, while only a minimal effect is exerted by the PSE-like conditions (Qiao et al., 2002), the occurrence of muscle myopathies and abnormalities significantly altered the proximate composition of meat thus affecting its nutritional value. Indeed, if compared to their unaffected counterparts, abnormal muscles exhibited an overall higher amount of moisture, fat, and collagen to the detriment of protein content (Kuttappan et al., 2012; Soglia et al., 2016a; Soglia et al., 2016b; Zambonelli et al., 2016). Besides, not only a threefold increase in energy deriving from fat but also elevated collagen-to-total protein ratio led to a significantly lowered nutritional value of severe white-striped meat (Petracci et al., 2014; Mudalal et al., 2014). Overall, these differences are likely ascribed to the progressive myodegeneration and regenerative processes, resulting in fibrosis and lipidosis, typically observed within the abnormal muscles. Indeed, while an increased fat (lipidosis) and connective tissue (fibrosis) deposition might, respectively, account for the higher fat and collagen content, the remarkably elevated

moisture level might be attributed to the moderate-to-severe edema resulting from the inflammatory processes (Petracci et al., 2014; Sihvo et al., 2014; Soglia et al., 2016a). Hence, both myodegeneration and the presence of histological lesions may have led to the extremely reduced protein content observed within the abnormal muscles (Petracci et al., 2014; Soglia et al., 2016a). With regard to mineral content, consistent with the mechanism leading to Duchenne muscular dystrophy in mammals, increased ion levels and alterations in sodium and calcium homeostasis were observed and associated with the development of muscle damage thus promoting the occurrence of muscular abnormalities (Sandercock and Mitchell, 2004; Wallace and McNally, 2009; Soglia et al., 2016a). Wooden breast muscle also exhibited lower content of anserine and carnosine are which are extremely important in the homeostasis of contractile muscles as a result of their role in buffering, antioxidation, and anti-lycation (Sundekilde et al., 2017). With regard to fatty acids profiles, the occurrence of muscle abnormalities led to significantly higher amounts of linoleic acid (Soglia et al., 2016b) previously found in *mdx* mice and positively correlated with sarcolemmal damage and oxidative stress as well (Tuazon and Henderson, 2012). However, although as a whole the total poly- and monounsaturated fatty acids contents were found to be similar in pectoral muscles from normal and dystrophic chickens, the last exhibited an overall reduction in $\Delta 5$ and $\Delta 6$ desaturase activity leading to lower amounts of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and other intermediate products from elongation and desaturation (Jordan et al., 1964; Soglia et al., 2016b). Interestingly, in spite of their significantly higher fat content, a lower amount of saturated fatty acids was associated with the occurrence of muscle abnormalities (Kuttappan et al., 2012; Soglia et al., 2016b). Considering these findings and the relatively low amount of heme pigments observed within the abnormal *P. major* muscles, the potential pro-oxidant activity of heme-iron released from the globin molecule of a damaged porphirin ring and the contextual exposure of phospholipids resulting from the structural changes associated with the severe myopathic lesions was hypothesized to affect oxidative stability (lipid oxidation and protein carbonylation level) of meat (Soglia et al., 2016a). As a consequence of the physical and chemical changes (including protein aggregation, polymerization, and intra- and inter-molecular cross-linking) induced by oxidation (Davies et al., 1987; Fagan et al., 1999; Lund et al., 2007; Kim et al., 2010) an overall decrease in protein solubility and functionality was observed within the abnormal muscles (Mudalal et al., 2014; Bowker and Zhuang, 2016).

With regard to meat quality traits, altered color and ultimate pH values were observed within the *P. major* muscles affected by abnormalities. Indeed, as a direct consequence of the strong fibrotic response and the lower amount of heme pigments, increased yellowness and pale color might be observed in abnormal muscles. When compared to their unaffected

counterpart, the affected cases revealed a remarkably higher ultimate pH values which, although associated with a lower glycogen content (Mutryn et al., 2015), were hypothesized to arise from a change in glucose utilization rather that in its availability (Zambonelli et al., 2016; Abasht et al., 2016). Indeed, unexpectedly, although several factors suggest the occurrence of hypoxic conditions, there was not an expected increased conversion of pyruvate into lactate (Zambonelli et al., 2016). Even if high ultimate pH values might significantly increase water-holding and processing attitudes of meat, since microbial growth strongly depends on pH, they may compromise the microbiological stability of meat (Barbut et al., 2008). Within this context, it seems reasonable to hypothesize that microbial shelf-life of meat affected by muscle abnormalities could be remarkably reduced as a consequence of their higher ultimate pH values. Moreover, in spite of the higher pH_u that should result in improved water-holding capacity of meat, the pectoral muscles affected by muscular abnormalities exhibited severely impaired technological properties (marinate uptake, cooking loss, and yield), as shown in Table 3.5. Indeed, reduced water-holding and water-binding capacities are associated with the occurrence of muscle abnormalities and likely linked to an overall reduction in protein functionality, with a more pronounced effect being exerted by the wooden breast rather that the white-striping defect (Mudalal et al., 2014; Mudalal et al., 2015; Bowker and Zhuang, 2016). This phenomenon might be partly due to protein aggregation and cross-linking following oxidation (Soglia et al., 2016b) and to the overall substantial reduction and altered profile of muscular contractile and sarcoplasmic proteins typically observed within the abnormal muscle tissues (Mudalal et al., 2014; Soglia et al., 2016a; Bowker and Zhuang, 2016). Dealing with that, the overall impairment of the water holding capacity of meat was corroborated by NMR relaxation properties examining both the relative intensity and the T_2 transverse relaxation time for the three proton populations (bound, intra-, and extra-myofibrillar water) identified within the muscle tissue. According to our previous study, the remarkable increased proportion and mobility of the extra-myofibrillar water fraction (the potential drip of the meat) may account for the lower water-holding ability of the abnormal cases during processing and storage (Soglia et al., 2016b).

The occurrence of muscle abnormalities not only alters the visual appearance of meat, impairing consumer acceptance (Kuttappan et al., 2012), but also significantly affects its textural properties. Overall, regardless of freshness, cooking, and the degree of abnormality, the textural properties of meat, are severely affected by the occurrence of muscle myopathies and abnormalities (Petracci et al., 2013b; Mudalal et al., 2015; Soglia et al., 2016a; Chatterjee et al., 2016). However, since extensive poor cohesion (fiber bundles separation) frequently affected the white-striped areas, textural differences were more pronounced with the occurrence of wooden breast rather than white-striping abnormality (Petracci et al., 2013b; Mudalal et al.,

2015). When compared to their unaffected counterparts, abnormal muscles exhibited elevated compression and Meullenet–Owens razor shear total energy (MORSE) forces as well as increased hardness and chewiness in the case of raw and cooked meat, respectively (Petracci et al., 2013b; Mudalal et al., 2015; Soglia et al., 2016a; Chatterjee et al., 2016). These changes in textural properties of meat might be explained by the profound alterations affecting the muscle fiber itself as well as the reduced water-holding capacity of meat leading to muscle shrinkage and increased packing density of fibers following cooking (Wattanachant et al., 2004; Huff-Lonergan and Lonergan, 2005). On the other hand, the thermally labile cross-links composing the newly deposed connective tissue might contribute to explain the absence of significant differences in the shearing properties of cooked unaffected and affected muscles (Mudalal et al., 2015). Because of undesired appearance and sensory properties, at least breast fillets with severe abnormalities are downgraded by visual evaluation and diverted to manufacture processed products where implications on sensory properties are of less importance. As a consequence, there is interest in developing automated systems to detect abnormal fillets (Yoon et al., 2016).

3.4 CONCLUSIONS

The genetic selection carried out within the past decades on broiler chickens in order to achieve increased growth rate and breast yield has promoted the development of several muscular myopathies and abnormalities. As a consequence, since the affected meat is normally downgraded and devoted to further processing, the occurrence of muscular abnormalities is associated with ever-increasing economic losses no longer sustainable by the poultry industry. In addition, not only decreased nutritional properties but also impaired sensory and technological quality traits have been observed. Within this context, with consumers being more sensitive to animal welfare, the ever-increasing incidence of muscle abnormalities may also negatively affect consumers' attitudes toward poultry meat.

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Chapter 4

Developments in Our Understanding of Water-Holding Capacity

Brian Bowker
United States Department of Agriculture, Athens, GA, United States

4.1 INTRODUCTION

Water-holding capacity (WHC) refers to the ability of meat to hold inherent or added moisture throughout fabrication, processing, and storage. For consumers, poor WHC in fresh poultry-meat products results in diminished visual appeal due to excess purge in packages and inferior palatability traits related to juiciness and tenderness. For processors, improved WHC in raw poultry meat leads to greater marinade pick-up and retention, better protein functionality, and greater processing and cooking yields.

Over the last several decades, the proportion of poultry marketed as whole intact carcasses has declined due to increased consumer demands for cut-up carcass parts and further-processed poultry products. To meet changing market demands, modern bird lines have been intensely selected for growth rate and carcass parts yields and an increasing percentage of birds are raised to heavier weights. In the United States, there has been an increasing demand for boneless, skinless poultry products. Unfortunately, problems with meat WHC are more readily apparent in these types of products compared to whole carcasses. Intrinsic changes within the birds themselves have also led to increasing problems with WHC. Although remarkable gains have been achieved in live production and carcass yield traits, modern birds seem to be more stress-susceptible and prone to developing myopathies that have a detrimental effect on WHC and other meat-quality traits (Petracci and Cavani, 2012).

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WHC in fresh poultry meat is a complex trait that is controlled by the chemical and structural attributes of the muscle tissue as they are influenced by the postmortem transformation of muscle to meat. The primary focus of this chapter is on the basic underlying structural and biochemical mechanisms that control WHC attributes in fresh poultry meat. Numerous interacting antemortem and postmortem factors influence WHC in the final product. Although genetics and nutrition certainly play a role in WHC through their influence on muscle growth, composition, and physiology; factors related to the time period immediately pre- and post-slaughter seem to have an overriding impact on WHC. Therefore, this chapter will only cover factors that influence WHC characteristics in fresh poultry meat from preslaughter activity through initial slaughter and processing.

4.2 METHODS FOR MEASURING WHC

The ability of fresh poultry to retain moisture is a complex trait and various measurement techniques are used to show different aspects of WHC. The most commonly used techniques for assessing WHC typically involve measuring weight changes in meat samples due to moisture loss caused by gravimetric forces, the application of external mechanical forces, or cooking. In the interpretation of WHC data, it is important to account for both the limitations of the testing method and the loss of moisture from the meat due to prior sample handling and processing. Although the different measures of WHC are often correlated, the relationships are not strong enough to allow a single test to be used as a consistent predictor of WHC in fresh poultry.

4.2.1 Gravimetric Methods

With gravimetric methods, WHC is determined by measuring the weight loss of a meat sample during storage without the application of external forces to the meat (Honikel, 2009). Fluid that is passively lost due to the force of gravity on the extracellular water in the muscle tissue serves as a good indicator of potential exudate accumulation during raw meat storage. The amount of weight loss is usually expressed as a percentage of the original sample weight and is commonly referred to as drip loss. Although many variations exist in the application of this method, drip loss in fresh poultry is usually measured by removing a standard-sized subsample of the muscle, suspending it in a sealed plastic bag or container which is then stored at 2−4°C for at least 24 h. After storage the meat sample is blotted to remove excess surface moisture and reweighed. Storage duration for the determination of drip loss typically ranges from 1 to 7 days. Variations in subsample size, surface area to weight ratio, and muscle fiber orientation can influence drip loss measurements. Drip loss can also be determined using intact poultry muscles.

4.2.2 External Mechanical Force Methods

WHC can also be determined by measuring the weight loss of a standardized meat sample due to the application of a set amount of external mechanical force. Exerting an external force on the meat samples accelerates fluid losses from the tissue and reduces assay time requirements. Fluid loss from the meat due to the application of external forces may reflect the loss of both extracellular and loosely bound intracellular water, depending upon the magnitude and duration of the force applied. The two most common methods used are the filter paper press method (Kauffman et al., 1986) and the centrifugal forces method (Honikel, 2009). With the filter press method, a meat sample of given size and geometry is placed on a preweighed piece of filter paper and compressed between two parallel plates using a specified pressure for a set amount of time. Uniform pressure is usually applied using a texture analyzer, a hydraulic press, or merely a weight. With the centrifugal forces method, a meat sample is placed in a centrifuge tube and g-forces are exerted on the sample using centrifugation. For both the filter press and centrifugal forces methods, wide experimental variations in sample size/geometry, applied forces, temperature, and time duration have been reported. Data are often reported as expressible moisture or even more simply as WHC. However, depending upon the specific method of calculation (fluid weight loss or sample weight retained), larger values can be indicative of either high or low WHC.

4.2.3 Cooking Methods

In fresh poultry, WHC is often determined by measuring the weight loss of meat due to cooking. Values are typically expressed as either cook loss or inversely as cook yield percentages and serve as good indicators of the production yield of cooked poultry meat products. Cook loss primarily reflects the loss of extracellular and intracellular water from the muscle tissue due to thermally induced protein denaturation and cell membrane disintegration but can also be influenced by the melting of lipids during cooking. Cook loss can be measured on whole intact muscles, subsamples, or even ground poultry meat. Samples are typically cooked to an internal temperature of 75−80°C either by roasting in a convection oven, steam cooking, or cooking in plastic bags placed in a heated water bath.

4.2.4 Other Methods

Aspects of WHC related to moisture-enhancement and freezing-thawing are also commonly reported in poultry meat. Salt-induced water uptake measurements are used to assess the ability of fresh poultry meat to hold added salt water, which is an indicator of the potential ability of the meat to pick up and retain marinade in moisture-enhanced products. For this assay, cold salt

brine (usually 0.6 M NaCl) is mixed with chopped meat, and then held at refrigerated temperatures for a set time before centrifuging and pouring off unbound liquid (Wardlaw et al., 1973). The addition of salt causes the myofibrils within the muscle cells to swell laterally and take up water (Offer and Trinick, 1983). Oftentimes the swollen meat pellet that remains after the salt-induced water uptake assay is heated in a water bath and then reweighed as a measure of cooking loss. The amount of fluid (or purge) that is expressed from meat upon thawing from a frozen state (i.e., thaw loss) is also used as an indicator of WHC in poultry. Thaw loss measurements, however, are highly variable and can be influenced by freezing and thawing rates and prefreezing moisture losses from the meat. Most of the accepted methods for measuring WHC in fresh poultry are both time-consuming and destructive to the samples. With varying levels of success, advanced technologies such as visible and near infrared spectroscopy (Prieto et al., 2009; Samuel et al., 2011; Bowker et al., 2014a; Hawkins et al., 2014), Raman spectroscopy (Herrero, 2008; Phongpa-Ngan et al., 2014), dielectric spectroscopy (Zhuang et al., 2007; Samuel et al., 2012), hyperspectral imaging (Elmasry et al., 2012), and nuclear magnetic resonance (NMR) relaxometry (Bertram et al., 2002a; Bertram and Ersen, 2004; Huang et al., 2014) have been investigated as rapid, nondestructive methods for predicting WHC.

4.3 MUSCLE COMPOSITION AND STRUCTURE

By weight, water is the most abundant component of fresh poultry meat. Lean muscle is comprised of approximately 75% water, 18%−20% protein, 2%−5% lipids, 1.5% nonprotein nitrogen substances, 1% carbohydrates, and 1% inorganic constituents (Offer and Knight, 1988). In living muscle, water functions as the medium for chemical reactions and metabolic processes within the tissue. Water enables the transportation of nutrients, metabolites, hormones, and waste in and out of the muscle cells. As a result of its biological functions, water is the principal component of the intracellular and extracellular fluid in muscle tissue and numerous chemical constituents are dissolved or suspended in it.

The WHC attributes of meat are strongly influenced by the complex, highly organized structure of skeletal muscle tissue. Skeletal muscle tissue is made up of elongated, multinucleated muscle cells (fibers) that are grouped together into muscle bundles (fasciculi). Individual muscle fibers are surrounded by the endomysium, a thin layer of connective tissue that lies above the sarcolemma (muscle cell membrane). Fasciculi are sheathed in a layer of connective tissue known as the perimysium. The entire muscle is surrounded by the epimysium, a thick layer of connective tissue that is usually continuous with the tendons that attach the muscle to bone.

Approximately 85% of the volume of a muscle fiber is taken up by myofibrils. Myofibrils are organelles that are long linear strands of repeating

contractile units called sarcomeres. A sarcomere is the basic contractile unit of muscle and is made up of overlapping thick and thin filaments that lie parallel to the muscle fiber. The thick and thin filaments account for the striated banding pattern viewed in muscle fibers under a microscope. The protein-dense A-band region of the sarcomere spans the length of the thick filaments and includes the area of overlap between the thick and thin filaments. The less protein dense I-band region of the sarcomere refers to the zone of the thin filaments that does not overlap with the thick filaments. The Z-lines designate the lateral borders of the sarcomeres and are viewed as dark lines in the middle of the I-bands. Within the Z-lines, thin filaments from adjacent sarcomeres are crosslinked by α-actinin molecules. Thin filaments consist primarily of actin, which is made up of globular molecules (~42 kDa) bound together to form a filamentous protein. The thin filament also contains proteins (troponin and tropomyosin) that regulate the interaction between the thick and thin filaments during muscle contraction. Thick filaments consist primarily of myosin, a large protein (~520 kDa) made up of six subunits (two ~220-kDa heavy chain subunits, two pairs of ~17- to 22-kDa light chain subunits). The filamentous tail regions of approximately 200 myosin molecules aggregate together to form the backbone of a thick filament while the globular head regions of the myosin molecules extend from the thick filament to interact with the surrounding thin filaments. The globular head of the myosin molecule has both actin binding and adenosine triphosphate (ATP)-binding sites.

During muscle contraction, Ca^{2+} is released from the sarcoplasmic reticulum into the sarcoplasm where it binds to troponin causing tropomyosin to shift. This in turn allows an actomyosin crossbridge to be formed between the head of the myosin molecule and an actin molecule in the thin filament. The myosin head bound to the actin molecule then tilts which pulls the thin filament toward the center of the sarcomere. The hydrolysis of ATP by the myosin provides the energy necessary for the swivel of the myosin head. The actomyosin crossbridge is then broken when the myosin head binds a new molecule of ATP. The repeated breaking and reformation of the actomyosin crossbridges between the thick and thin filaments is the basis of sarcomere shortening and muscle contraction.

Numerous cytoskeletal proteins embedded within the sarcomeres and around the periphery of the myofibrils help to maintain myofilament structural integrity, preserve myofibril alignment within the cell both laterally and longitudinally, and provide a linkage between the myofibrils and the cell membrane. Titin is a large filamentous protein (~3700 kDa) with elastic regions that extends longitudinally from the Z-line to the M-line and is vital to keeping thick filaments aligned and maintaining sarcomere integrity. Nebulin is a filamentous protein that runs along the thin filament and binds to actin and α-actinin to provide stability to the thin filament. Myofibrils are laterally connected to adjacent myofibrils by a proteinaceous network of

intermediate filaments around the Z-disk. The intermediate filaments are comprised of numerous fibrous proteins including desmin, filamin, vimentin, and synemin. Costameres are Z-line-associated structures that connect peripheral myofibrils to the cell membrane and are comprised of multiple proteins including dystrophin, talin, and vinculin. The costameres play a key role in transmitting force generated within the sarcomere to the sarcolemma and extracellular matrix. Thus, the highly organized structure of the muscle cells and the integrated multitiered connective tissue within the muscle tissue allows the force generated within sarcomeres and muscle fibers to be efficiently transmitted to the entire muscle to generate movement of the limb or attached structure.

4.4 CONVERSION OF MUSCLE TO MEAT

During the conversion of muscle to meat, the chemical and physical changes that occur in muscle tissues immediately before and after birds are slaughtered profoundly influence the final WHC characteristics of poultry meat. Due to blood removal (exsanguination) and the subsequent lack of oxygen supply to the tissue, the muscle cells shift from aerobic to anaerobic metabolism in an attempt to maintain cellular energy (ATP) levels and homeostasis. Once oxygen and phosphocreatine supplies are depleted in the tissue, muscle glycogen is broken down through anaerobic glycolysis to produce ATP and lactic acid. Compared to aerobic metabolism, however, anaerobic pathways produce less ATP and as a result the postmortem muscle is unable to sustain muscle energy levels. As ATP levels decline to about one-third to one-half of their original levels (depending upon temperature), Ca^{2+} begins to leak out of the sarcoplasmic reticulum triggering the formation of actomyosin crossbridges, muscle contraction, and the onset phase of rigor mortis. In chicken and turkey muscle, the delay time before the onset of rigor mortis is typically <0.5 h and <1 h, respectively. Once ATP concentrations within the muscle reach critically low levels, actomyosin bonds cannot be broken, the muscle becomes inextensible, and rigor mortis is complete. Rigor mortis completion normally takes 4–6 h in chickens and 6–8 h in turkeys.

A by-product of anaerobic glycolysis in postmortem muscle is lactic acid. As the circulatory system is no longer able to remove metabolites from postmortem muscle, lactic acid accumulates and pH declines in the muscle until glycogen is depleted or glycolysis is stopped by the low pH. During the postmortem period, muscle pH declines from physiological levels of pH 7.0 to an ultimate meat pH of 5.7–5.9 by approximately 6–8 h postmortem in chickens and turkeys. The rate and extent of postmortem pH decline can vary widely between muscles and carcasses and strongly influences WHC in fresh poultry meat.

Because they are both linked to energy metabolism, postmortem muscle pH decline and rigor development are closely related to each other. In

muscle with a rapid pH decline early postmortem, the ability of the tissue to regenerate ATP quickly decreases because energy supplies (glycogen) are rapidly depleted and the low pH prevents further glycolysis and ATP regeneration. As a result, rigor onset and completion occur earlier in muscles with a rapid postmortem pH decline. In muscles where the postmortem pH decline is abbreviated due to initial energy supplies within the muscle, rigor onset and completion also occur earlier. Thus, rigor mortis takes longer to develop in muscles with an intermediate pH decline compared to muscles with a rapid or limited postmortem pH decline.

As postmortem glycolysis proceeds and cellular homeostasis is disrupted, the microenvironments of the muscle fibers change. Due to ATP-dependent sodium, potassium, and calcium pumps losing their ability to function properly, muscle fibers have a diminishing capacity to maintain reducing conditions within the cells and an increasing ionic strength. Alterations to fiber membrane integrity and permeability also occur postmortem. Although these postmortem changes likely influence the ability of the muscle fibers to retain and bind water, their direct contributions to WHC in fresh poultry meat have not been thoroughly researched.

During the conversion of muscle to meat, changes in muscle fiber size and spacing occur. With postmortem time and rigor development, muscle fibers shrink in diameter (Hegarty, 1970; Swatland and Belfry, 1985; Offer and Cousins, 1992) and the extracellular space around the fibers increases (Hegarty, 1970; Swatland and Belfry, 1985; Offer and Cousins, 1992). These changes likely have a strong influence on WHC as the shrinkage of the muscle fibers leads to the development of gaps between the muscle fibers and between fiber bundles during the postrigor period through which water can easily escape from the tissue (Offer and Trinick, 1983; Offer and Cousins, 1992; Offer et al., 1989).

4.5 DISTRIBUTION AND TYPES OF WATER WITHIN MUSCLE

Due to the highly organized structure of muscle, there are several compartments within the tissue where water is held. The majority of the water is contained intracellularly within the muscle fibers. Approximately 85% of water in muscle is considered intra-myofibrillar, as it is held within the spaces between the thick and thin filaments of the myofibrils (Offer and Knight, 1988). The remaining portion of the water in muscle is held in the intracellular space between the myofibrils, the intracellular space between the myofibrils and the sarcolemma, or in the extracellular space within and between the muscle fiber bundles (Offer and Cousins, 1992). The proportion of water in these various compartments and the mobility of water between these compartments strongly influence WHC attributes in fresh poultry meat. Water exists in muscle in three forms: bound, immobilized, and free (Fig. 4.1). These categories vary widely with regards to how strongly the

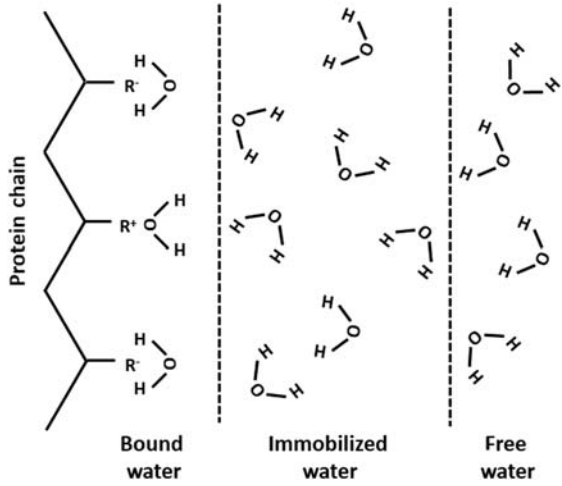


FIGURE 4.1 Three forms of water in muscle. The bound water layer (left) consists of water molecules that are tightly bound to the charged hydrophilic groups of the muscle proteins in a polarity-dependent manner. The immobilized water layer (middle) consists of water molecules that are held by steric effects within the muscle structure and by weak attractions to bound water and muscle proteins. Free water molecules (right) are only held within the muscle by weak capillary forces.

water is retained within the structure of the muscle and influence the degree to which water can be lost from the meat.

4.5.1 Bound Water

Water is a polar molecule with the oxygen atom having a slight negative charge and the two hydrogen atoms having slight positive charges. Due to its dipolar nature, water is attracted to the charged hydrophilic amino acid side chains of muscle proteins. In muscle, the fraction of water that electrostatically interacts with the muscle proteins is referred to as bound water. The amount of water tightly bound to proteins represents only a small proportion of the total water in muscle. It has been estimated that only about 0.5 g of water per gram of protein is tightly bound to muscle proteins and that this inner layer of water represents less than a tenth of the total water in muscle (Huff-Lonergan and Lonergan, 2005). Bound water does not move to other compartments within the muscle. Due to its tight binding to proteins and reduced mobility, bound water is not easily lost from fresh meat and the amount of bound water remains essentially constant in postrigor muscle (Offer and Knight, 1988). Bound water is resistant to freezing and conventional heating and is generally not influenced by meat processing (Fenema, 1985). Thus, bound water has little influence on the observed WHC attributes in fresh and processed poultry.

4.5.2 Immobilized Water

Additional water molecules are subsequently attracted to the bound water in layers by forces that become successively weaker as the distance from the reactive groups on the proteins increases. Immobilized or entrapped water refers to the fraction of water that is held by steric effects within the muscle structure and by moderate to weak attractions to bound water and muscle proteins (Fenema, 1985). This water is located within and between the thick and thin filaments of the myofibrils (Honikel et al., 1986) but has weaker associations with the muscle proteins and other cellular components than bound water. Immobilized water can account for up to 85% of the total water in muscle. Unlike bound water, immobilized water in meat can be strongly influenced by processing. Immobilized water does not readily leak from the tissue early postmortem, but it is susceptible to being lost due to cooking or freezing. Immobilized water in the muscle tissue is strongly affected by the intrinsic alterations that occur in the muscle cell structures and pH during the postmortem transformation of muscle to meat and throughout storage. Changes to the structural components of the muscle fibers (myofibrils, cytoskeletal linkages, and membranes), the net charge of myofibrillar proteins, and the amount of extracellular space within the muscle can drastically influence the retention of the immobilized water fraction. Water that moves from the immobilized to the free category due to changes in the tissue can be easily lost from the meat as purge or drip. Thus, the retention of immobilized water within the intra-myofibrillar spaces is crucial for maintaining good WHC in fresh poultry meat.

4.5.3 Free Water

Water whose flow from the muscle tissue is only restricted by cell membranes and capillary forces is referred to as free water. Free water is not readily observed in meat prior to rigor mortis. The formation of permanent actomyosin crossbridges can cause entrapped water to be mobilized out of the myofibrillar structures. In general, rigor mortis causes the amount of immobilized water to decrease and the amount of free water to increase. As free water is only held in the meat by weak surface forces, it is easily released from the tissue as drip due to physical forces that damage muscle cell integrity. Thus, free water is easily lost through common meat processing and handling steps such as grinding, cutting, cooking, and storage.

4.6 UNDERLYING MECHANISMS THAT INFLUENCE WHC

During the transformation of muscle to meat, numerous interacting biochemical and structural changes occur within the muscle tissue. Controlling the location of the inherent or added water in the muscle tissue and retaining

moisture throughout these postmortem changes is important for maintaining WHC and optimizing product yield. Postmortem changes in the muscle tissue influence WHC attributes in fresh poultry meat through net charge and steric effects. Changes in the degree of postmortem protein denaturation and degradation in the muscle tissue are also thought to influence WHC.

4.6.1 Net Charge Effects

The interactions between meat pH and the net electrical charges on the muscle proteins strongly influence WHC (Fig. 4.2). As pH in postmortem muscle declines due to the accumulation of lactic acid, there is an overall reduction in the availability of reactive groups on the muscle proteins for binding water. The isoelectric point (pI) of a muscle protein is the pH at which the number of negative and positive charges on the protein are essentially equal and the overall net charge of the protein is zero. At this pH, the positive and negative charged groups within a protein attract each other and the ability of the protein to attract and hold water is minimal. Because myosin and actin are the predominant proteins within muscle, their pI's (approximately 5.1−5.4) represent the pH at which the WHC of meat is minimal. The further meat pH is above the pI of the predominant muscle proteins, the

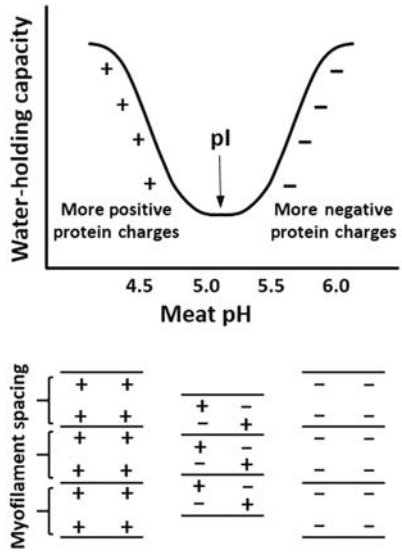


FIGURE 4.2 Net charge effect on water-holding capacity. Meat pH influences the distribution of charges on muscle proteins and myofilament spacing. At the isoelectric point (pI), the net charge on the muscle proteins is zero, myofilament spacing is reduced, and water-holding capacity is at its minimum. At meat pH values above or below the pI, similar charges on the muscle proteins repel each other causing an increase in myofilament spacing and water-holding capacity.

stronger the protein charge and the greater the WHC. Thus, minor changes in fresh poultry meat pH can lead to great changes in WHC.

4.6.2 Steric Effects

The primary factor controlling WHC in fresh poultry is steric (or spatial) effects within the myofibrils. As much as 85% of the water in muscle is held within the myofibrils by capillary forces generated from the arrangement of the thick and thin filaments. As the sarcomeres are essentially isovolumetric during contraction and relaxation, the amount of fluid contained within myofibrils is relatively constant in living muscle cells (Millman et al., 1983). In postmortem muscle, however, the location of intra-myofibrillar water is influenced by postmortem changes within the tissue. Both ultimate meat pH and rigor mortis development strongly impact WHC attributes in fresh meat due to their interacting influence on the amount of intra-myofibrillar space available for water binding.

The net charge effect in meat, which is controlled by postmortem muscle pH, not only influences WHC by altering the electrostatic attraction between muscle proteins and water but also plays a structural role. As the net charges on the myofibrillar proteins near zero as the meat pH approaches the pI, the electrostatic repulsion between the myofilaments is reduced. As a result, the structures within the myofibrils pack more closely together and reduce the space available within the myofibril to hold water (Fig. 4.2). Using X-ray diffraction measurements in pork, it has been shown that with a drop in ultimate meat pH from 6.4 to 5.2, the distance between the myosin filaments can decrease from approximately 46 to 40 nm in linear proportion to pH (Diesbourg et al., 1988; Irving et al., 1990). This lateral decrease in myofilament spacing is sufficient to force fluid out of the myofibrils into the surrounding extramyofibrillar space.

Rigor mortis impacts the amount of water held within the myofibrils and ultimately WHC due to the longitudinal shrinkage it causes to the myofibrillar structure (Fig. 4.3). During rigor development, sarcomeres can shorten causing an increased overlap of the thick and thin filaments and a decrease in I-band width. Studies using low-field NMR relaxometry have shown that the I-band region of the sarcomere structure contains a greater proportion of the water within a myofibril than the more protein-dense A-band region (Bertram et al., 2002b). These data suggest that the predominant space available for holding water within myofibrils is decreased by rigor shortening, which causes a reduction in the volume of the I-band region of the sarcomere. Thus, longitudinal shortening of the sarcomeres reduces the intramyofibrillar space available for holding water and forces fluid into the extramyofibrillar portion of the muscle cells where it can more easily migrate to the extracellular space and be lost from the meat as drip (Bertram et al., 2002b; Bendall and Swatland, 1988). This hypothesis is consistent

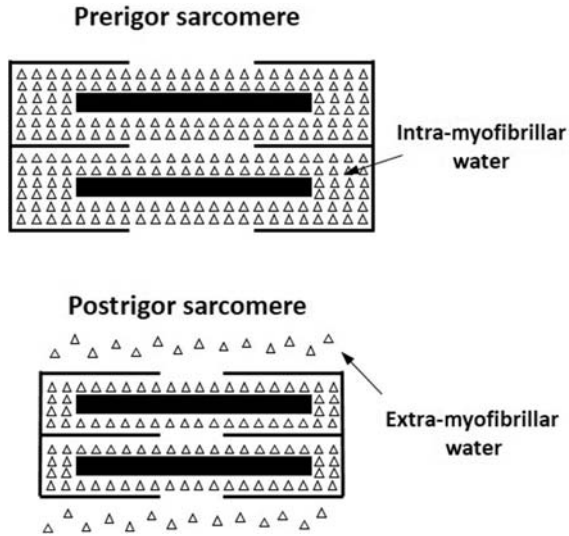


FIGURE 4.3 Steric effects on water-holding capacity. Rigor mortis development causes sarcomeres to shrink both longitudinally and transversely. This shrinkage reduces the intramyofibrillar space available for holding water and forces water out of the myofibrils. Water in the extramyofibrillar portions of the muscle cells can migrate to the extracellular spaces where it is more easily lost from the meat as drip.

with the observation that drip loss increases linearly as sarcomere length decreases in pork (Honikel et al., 1986). In poultry muscles, the degree of sarcomere shortening during rigor development depends on intrinsic differences in tension put on the muscle due to anatomical positioning and attachment as well as processing factors such as deboning time.

The transverse shrinkage of myofibrils due to rigor development may also influence WHC in fresh meat (Fig. 4.3). During normal muscle contraction in living tissue only about 20% of potential actomyosin crossbridges form. Upon rigor completion in postmortem muscle, however, more than 90% of potential actomyosin crossbridges (rigor bonds) are formed in the areas where the thin and thick filaments overlap (Aberle et al., 2001). The formation of these permanent actomyosin crossbridges not only causes a significant reduction in the spacing between the myofilaments, but also prevents the lateral expansion of the myofibrils when the sarcomeres shrink longitudinally during rigor development (Swatland and Belfry, 1985; Bendall and Swatland, 1988). As a result, water held within the myofilament lattice is forced out of the myofibrils and into the extramyofibrillar space during rigor development. Thus, rigor mortis influences WHC in fresh poultry meat due to its effects on both the longitudinal shrinkage of myofibrils during sarcomere shortening and the transverse spacing between the thick and thin filaments of the sarcomere.

Steric effects play a key role in determining WHC attributes in fresh poultry meat by influencing the distribution of fluid between the intramyofibrillar and extramyofibrillar compartments of the muscle cells. Rigor development and postmortem muscle pH influence both the lateral and longitudinal shrinkage of the sarcomere structure and control the volume of space available between the myofilaments for holding water. As more fluid from inside the myofibrils is forced into the extramyofibrillar space, it can migrate into the extracellular space where it is more easily lost from the muscle tissue during processing, storage, and handling. Thus, the rate and quantity of drip formation in fresh poultry meat is thought to be influenced by both the degree of rigor shortening and cell membrane permeability. Because rigor development and myofibril shortening are typically complete by 4–6 h postmortem in poultry breast muscle, it has been postulated that the amount of extracellular water reaches its peak value at this postmortem time and that sarcomere shortening does not force intracellular fluid out of the cells after 4–6 h postmortem (Zhuang and Savage, 2012).

4.6.3 Protein Denaturation

Changes in the functional properties of muscle proteins due to denaturation during the conversion of muscle to meat influence WHC in fresh poultry. Myosin is the most abundant protein in muscle and plays a significant role in WHC. In PSE (pale, soft, exudative) pork, increased postmortem myosin denaturation is closely associated with inferior WHC attributes. The PSE condition is typically caused by an accelerated muscle metabolism early postmortem and a rapid pH decline. As a result, muscle proteins are exposed to low pH conditions while muscle temperatures are still elevated which leads to increased denaturation. The denaturation of native myosin heads under pH and temperature conditions similar to PSE causes the lengths of the myosin heads to shrink from 19 to 17 nm (Offer, 1991). This myosin head shrinkage causes the thick and thin filaments to draw closer together during rigor development. Thus, postmortem myosin denaturation is thought to diminish WHC by reducing myofilament lattice spacing which causes additional fluid to be expelled from the intramyofibrillar space.

Poultry breast muscle is particularly susceptible to yielding pale-color meat with poor WHC characteristics. Similar to PSE pork, the poor WHC in PSE-like turkey breast meat is generally attributed to a rapid postmortem pH decline and extensive myosin denaturation. (Pietrzak et al., 1997; Molette et al., 2003; Molette et al., 2006). In chickens, however, postmortem myosin denaturation does not seem to be the main cause of the low WHC in PSE-like broiler breast meat. Several studies have shown that in broiler breast meat with widely divergent WHC attributes there are minimal differences in myofibrillar protein denaturation as measured by protein solubility (Van Laack et al., 2000; Bowker and Zhuang, 2015). These studies suggest that

low muscle pH, rather than myosin denaturation, may be the primary determinant of low WHC in PSE-like broiler breast meat. Species differences in the relationship between postmortem myofibrillar protein denaturation and WHC may be due to differences in rigor development and the inherent susceptibility of myosin to denaturation. In vitro investigations have suggested that myosin from chicken *pectoralis* muscle is more resistant to denaturation under postmortem muscle conditions than myosin from pork muscle (Van Laack and Lane, 2000). It has also been suggested that the degree of myosin denaturation is not only affected by the severity of the muscle pH and temperature conditions but also by the duration of exposure to these conditions (Offer, 1991). It has been estimated that approximately 10%−50% of myosin heads are denatured during rigor development depending on the rate of pH and temperature decline in the muscle (Offer, 1991). The formation of actomyosin bonds during rigor development protects the head of the myosin molecule from further denaturation (Bowker et al., 2005). Thus, it is postulated that the timing of rigor onset and the extent of overlap between the thick and thin filaments may play a role in the lack of myofibrillar protein denaturation observed in chicken breast meat. Further research is needed to fully delineate the relationship between myofibrillar protein denaturation and WHC in poultry.

Although sarcoplasmic proteins do not play a structural role within the muscle cells, postmortem sarcoplasmic protein denaturation seems to be related to WHC. In both chicken (Van Laack et al., 2000; Bowker and Zhuang, 2015) and pork (Lopez-Bote et al., 1989; Joo et al., 1999), the correlations between sarcoplasmic protein solubility and various measures of WHC are consistently greater than the correlations between myofibrillar protein solubility and WHC. Sarcoplasmic proteins such as phosphorylase, creatine kinase, triose phosphate isomerase, and myokinase are increasingly denatured in pork with low WHC (Joo et al., 1999; Warner et al., 1997). Similarly, increased phosphorylase denaturation has been observed in chicken (Bowker and Zhuang, 2015; Zhu et al., 2011) and turkey (Pietrzak et al., 1997; Rathgeber et al., 1999b) breast meat with low WHC. Increased creatine kinase denaturation has also been observed in turkey breast meat that exhibits rapid postmortem glycolysis (Rathgeber et al., 1999b).

Although a causal relationship between sarcoplasmic protein denaturation and low WHC has not been proven, the available data suggest that the denaturation of sarcoplasmic proteins may influence WHC by reducing the ability of the myofibrillar proteins to bind water. In vitro research has demonstrated that the addition of sarcoplasmic proteins to isolated myofibrils increases their WHC. However, if the sarcoplasmic proteins are denatured prior to addition to the myofibrils then WHC is diminished (Monin and Laborde, 1985). Electrophoresis and immunochemical data indicate that in turkey and chicken breast meat with poor WHC denatured sarcoplasmic proteins disappear from the water-soluble fraction of muscle and adhere to myofibrils

during subcellular fractionation (Pietrzak et al., 1997; Bowker and Zhuang, 2015; Zhu et al., 2011). Thus, it has been postulated that denatured sarcoplasmic proteins may coagulate onto myofibrils in postmortem muscle tissue and diminish WHC by altering myofibrillar surface interactions with water (Puolanne and Halonen, 2010). Further research is needed, however, to establish whether there is a mechanistic link between in vivo postmortem sarcoplasmic protein denaturation and WHC in fresh poultry meat.

4.6.4 Protein Degradation

Although the reductions in myofibrillar lattice volume due to pH and rigor strongly influence the amount of water retained within the myofibrils, these factors do not fully account for moisture loss from the tissue and WHC. The physical connections between myofibrils and between myofibrils and the cell membrane play a key role in determining the degree to which myofibril shrinkage causes reductions in overall muscle cell volume and the expulsion of fluid into the extracellular space where it can more easily be lost from the tissue (Kristensen and Purslow, 2001; Melody et al., 2004; Morrison et al., 1998). Myofibrils within the muscle cells are linked to each other by intermediate filaments and to the sarcolemma by costameres (Wang and Ramirez-Mitchell, 1983). Thus, the integrity of these cytoskeletal linkages strongly influences the transmittal of myofibril shrinkage to the entire muscle cell.

Many of the proteins that make up these cytoskeletal linkages are known to be susceptible to postmortem proteolysis during meat aging. In both red meat and poultry, myofibrillar and cytoskeletal proteins including desmin, titin, nebulin, talin, and vinculin are progressively degraded in postmortem muscle. The postmortem degradation of cytoskeletal proteins is generally attributed to the endogenous calpain system which consists of Ca^{2+}-dependent cysteine proteases (calpains) and their competitive inhibitor (calpastatin). The isozymes μ-calpain and μ/m-calpain present in poultry muscle exhibit greater calcium sensitivity than the calpains (μ-calpain and m-calpain) exhibited in mammals (Sorimachi et al., 1995; Lee et al., 2007; Lee et al., 2008b). This difference in calcium sensitivity likely accounts for the more rapid postmortem proteolysis and aging tenderization observed in poultry muscle (Lee et al., 2008b). The activity of the calpain system and its subsequent effects on cytoskeletal protein degradation are strongly influenced by postmortem changes in pH, ionic strength, and protein oxidation that occur in the muscle (Huff-Lonergan et al., 2010).

A growing body of evidence suggests that the postmortem movement of fluid into the extracellular spaces and ultimately WHC may be influenced by the degree of postmortem degradation of cytoskeletal proteins. In pork, it has been observed that improvements in WHC (as measured by gravimetric and centrifugal drip loss) are closely associated with increased postmortem proteolysis of intermediate filaments and costamere proteins such as desmin,

vinculin, and talin (Kristensen and Purslow, 2001; Melody et al., 2004; Morrison et al., 1998; Bee et al., 2007; Davis et al., 2004; Gardner et al., 2005). These data suggest that the degradation of cytoskeletal proteins early postmortem may improve WHC in fresh poultry meat by minimizing muscle cell shrinkage and reducing the flow of fluid into the extracellular space. Although this hypothesis is consistent with data on broiler breast meat that show a simultaneous increase in WHC and the extent of protein degradation between 8 and 24 h postmortem (Bowker and Zhuang, 2014), the general agreement in the postmortem time course of these events is not proof of this hypothesis. Further research is needed to more directly test and quantify the contribution of cytoskeletal protein degradation to variations in the WHC of fresh poultry meat.

4.7 PSE POULTRY MEAT

Poor WHC attributes in fresh poultry are often associated with PSE meat, particularly in the *Pectoralis major* muscle. The characteristics and causes of PSE-like meat in both chicken and turkey meat have been the subject of much research (Solomon et al., 1998; Barbut et al., 2008; Petracci et al., 2009; Petracci et al., 2015). Fresh poultry meat is often categorized as PSE based solely on having high L^* (lightness) color values and low pH. With regards to WHC attributes, PSE-like breast meat exhibits greater drip loss and cook loss, and lower marinade uptake (Van Laack et al., 2000; Barbut et al., 2005). The PSE condition was first observed in stress-susceptible swine that had altered Ca^{2+} regulation within the muscle due to a genetic mutation in the ryanodine receptor of the sarcoplasmic reticulum. In chickens and turkeys, a similar genetic mutation has not been proven (Strasburg and Chiang, 2009). The development of poor WHC attributes and PSE-like breast meat is primarily due to altered postmortem muscle metabolism and pH decline. In poultry, PSE-like breast meat typically develops due to an accelerated rate of muscle pH decline early postmortem ($pH < 6.0$ within 1 h postmortem) while carcass temperatures are still high. Breast muscle is particularly susceptible to developing poor WHC if there is a rapid pH decline and elevated muscle temperature due to excessive struggling and wing flapping immediately preslaughter or inadequate chilling due to its relatively large muscle mass compared to other parts of the carcass. Poultry meat with a normal rate of pH decline can also develop PSE-like characteristics if the muscle undergoes an extended postmortem pH decline resulting in lower ultimate pH ($pH_u < 5.8$).

4.8 MUSCLE FIBER TYPE DIFFERENCES

The water-binding attributes of poultry meat are partially a function of muscle fiber type composition. Though structurally similar, muscle fibers

vary in their contractile, biochemical, and metabolic properties due to the expression of different muscle protein isoforms. Although intermediate fiber types exist, muscle fibers are generally categorized into three groups based on contractile speed and metabolism: Type 1, slow-twitch oxidative fibers; Type IIA, fast-twitch oxidative/glycolytic fibers; and Type IIB, fast-twitch glycolytic fibers. Based on differences in visual appearance and myoglobin content, muscle fibers are generally referred to as red (Type I and IIA) and white (Type IIB) fibers.

Muscles are typically a heterogeneous mixture of fiber types. Muscles with predominantly white fibers have lower fatigue resistance, capillary density, abundance of mitochondria, and lipid content and greater average fiber diameter and glycogen content than muscles with mostly red fibers. Muscle fiber type composition differs among species, between muscles within a single bird, and can change depending upon the growth stage of the bird. Muscle fiber type composition controls the intrinsic metabolic profile of a muscle and can influence postmortem metabolism and WHC. White muscle fibers generally have a greater postmortem accumulation of lactic acid than red fibers due to their predisposition to generate ATP from muscle glycogen and the glycolytic pathway. Red muscle fibers are more equipped for oxidative metabolism and in poultry undergo a faster rate of rigor development under the anaerobic postmortem conditions. As a result, muscles with mostly white fibers such as the breast typically exhibit lower postmortem pH and inferior WHC compared to the red muscles of the leg and thigh. Because broiler and turkey *pectoralis* muscles contain nearly 100% type IIB glycolytic fibers, these muscles are highly susceptible to undergoing a rapid or extended postmortem pH decline and developing inferior WHC attributes.

4.9 ANTEMORTEM FACTORS

Although WHC attributes are a manifestation of biochemical and structural alterations that occur within the muscles after the birds are slaughtered, antemortem factors such as preslaughter activity and environmental conditions can influence postmortem changes in muscle tissue and WHC in the final meat product. During the 24-h period immediately prior to slaughter, birds are subjected to multiple events that may be stressful. During this time, birds typically undergo feed withdrawal, catching and cooping on the farm, transportation to and holding at the processing facility, and depending on the slaughter process, unloading and live shackling. Live bird management and handling practices as well as environmental conditions during this critical preslaughter time period can influence the physiological stress levels of the birds and the metabolic state of the skeletal muscles at the time of slaughter. These in turn can potentially affect the rate and extent of postmortem muscle metabolism and ultimately meat quality attributes including WHC.

4.9.1 Feed Withdrawal

In order to reduce carcass fecal contamination during slaughter processing, birds are typically held off feed for a period of time immediately prior to slaughter. Because prolonged preslaughter feed withdrawal (>12 h) in broilers reduces muscle glycogen content (Murray and Rosenberg, 1953; Shrimpton, 1960; Warriss et al., 1988; Kotula and Wang, 1994; Wang et al., 2013), it has been speculated that feed withdrawal may influence postmortem muscle glycolysis and WHC. Several studies have shown that feed withdrawal can lead to increased postmortem muscle pH (Warriss et al., 1988; Wang et al., 2013; Ngoka et al., 1982; Komiyama et al., 2008) and improved WHC (Kotula and Wang, 1994; Ngoka et al., 1982; Komiyama et al., 2008). In other studies, however, feed withdrawal did not influence postmortem muscle pH decline or WHC (Savenije et al., 2002a; Contreras-Castillo et al., 2007; Delezie et al., 2007). The varied effects of feed withdrawal on postmortem muscle may be dependent upon other antemortem factors that influence the physical activity and stress levels of the birds. Although feed withdrawal practices vary throughout the industry, for carcass contamination reasons it is recommended that feed be withheld from birds for 8−12 h prior to slaughter for broilers and 6−12 h for turkeys. Though limited, the available data suggests that the range of feed withdrawal times commonly used in the industry do not drastically alter ultimate meat pH or WHC attributes in fresh poultry.

4.9.2 Preslaughter Activities

The period of time from when birds are harvested on the farm to slaughter at a processing facility is one of intense activity that can potentially influence WHC attributes in the final product if birds are subjected to excessive stress. Prior to transportation to the slaughter facility, birds are manually or mechanically caught and placed in crates. Although bird handling and crating have been shown to increase plasma corticosterone and physiological stress levels in birds (Kannan and Mench, 1996; Nijdam et al., 2005), available evidence suggests that the method of catching (manual or mechanical) typically has little impact on final meat quality (Nijdam et al., 2005; Schilling et al., 2008). During warm summer months, however, lower drip loss, cooking loss, and expressible moisture (i.e., improved WHC) have been observed in breast meat from mechanically caught broilers (Schilling et al., 2008).

During transportation, birds can be exposed to numerous stressors such as adverse environmental conditions, crowding, motion, noise, and social disruptions (Freeman et al., 1984; Nicol and Scott, 1990; Mitchell and Kettlewell, 1998). Transportation is known to increase the physiological stress response in birds (Delezie et al., 2007; Freeman et al., 1984; Kannan et al., 1997b; Mitchell et al., 1992; Zhang et al., 2009; Xing et al., 2015) but

the direct effect of transportation stress on WHC is not well established. Breast meat from broilers subjected to short-term (0.5 h) transportation prior to slaughter has been shown to exhibit greater drip loss and cook loss (Xing et al., 2015) and a greater incidence of PSE-like characteristics (Oba et al., 2009) than breast meat from broilers undergoing longer transportation times (1−4 h). On the other hand, it has been reported that the transportation of turkeys for 3 h immediately prior to slaughter increased marinade retention and decreased cook loss in marinated breast meat but had no effect on the drip loss and cook loss of non-marinated meat (Owens and Sams, 2000). Most studies, however, have not observed direct transportation effects on broiler breast or thigh meat WHC attributes such as drip loss, cook loss, or expressible moisture (Savenije et al., 2002a; Delezie et al., 2007; Zhang et al., 2009; Debut et al., 2003; Doktor and Poltowicz, 2009). The varied effects of transportation on WHC are likely due to the interacting influences of transportation duration, feed withdrawal status, environmental conditions, and subsequent lairage conditions.

The influence of lairage on meat pH and WHC depends on prior transportation stress levels, environmental conditions, and duration (Zhang et al., 2009; Oba et al., 2009; Warriss et al., 1999; Guarnieri et al., 2004; Jiang et al., 2016b). Lairage prior to slaughter allows birds to recover from transport stress and has been shown to reduce the drip loss and cook loss in broiler breast meat (Jiang et al., 2016b) and to reduce the incidence of PSE-like meat (Oba et al., 2009). Furthermore, reducing heat stress on birds with water mist spraying and forced ventilation during lairage in the summer months has been shown to decrease drip loss and cook loss in broiler breast meat (Guarnieri et al., 2004; Jiang et al., 2016a).

The WHC attributes of fresh poultry meat can be influenced by the physical struggling and wing flapping of birds on the shackle line prior to slaughter. Shackling can be stressful to birds and can increase antemortem muscle glycolysis (Kannan et al., 1997a; Debut et al., 2005; Bedanova et al., 2007). It has been suggested that antemortem struggling and muscle glycogen content at the time of slaughter may be associated with the onset and extent of postmortem pH decline and ultimately meat quality (Debut et al., 2003; Berri et al., 2005). The use of anesthesia to eliminate the excitation and struggle prior to slaughter in turkeys results in postmortem breast muscle with higher initial pH and glycogen levels and increased WHC (Ngoka et al., 1982). These data suggest that efforts to minimize bird struggling during shackling generally improve WHC attributes in fresh poultry meat.

4.9.3 Environmental Conditions

Although preslaughter activities can negatively impact WHC if done improperly, adverse environmental conditions tend to have a greater impact on WHC than other antemortem factors. When birds are exposed to elevated

temperature and relative humidity conditions beyond their comfort level, they can experience heat stress as they lose the ability to efficiently dissipate heat. Heat stress in birds is typically associated with elevated environmental temperatures but it can also be associated with physical stressors such as crowding during crating, transportation, and lairage. Both acute and chronic heat stress are major sources of physiological stress to birds that can ultimately result in poultry meat with inferior WHC characteristics. Heat stress during the grow-out phase and preslaughter activity of birds has been shown to increase drip loss and cook loss in fresh meat from both broilers (Northcutt et al., 1994; Sandercock et al., 2001; Feng et al., 2008; Wang et al., 2009; Zhang et al., 2012) and turkeys (McKee and Sams, 1997). Seasonal data indicate that a higher incidence of meat with PSE-like characteristics and reduced WHC is typically observed during warm summer months compared to other times of the year in broilers (Petracci et al., 2004; Bianchi et al., 2007) and turkeys (McCurdy et al., 1996; Bianchi et al., 2004). Other studies have not observed a strong heat stress effect on breast meat quality suggesting that the effects of heat stress on WHC are likely dependent upon the intensity of the thermal stress and the interacting influence of other antemortem stressors (Debut et al., 2003; Petracci et al., 2001).

Antemortem heat stress can negatively impact WHC in poultry by inducing metabolic and physical changes within the living muscle tissue that ultimately influence the postmortem transformation of muscle to meat. Heat stress on birds can accelerate the rate of rigor mortis development and postmortem pH decline in breast muscle (Sandercock et al., 2001; Feng et al., 2008; Wang et al., 2009; McKee and Sams, 1997), which can reduce WHC. Heat stress in birds can lead to cell damage and Ca^{2+}-mediated increases in cell membrane permeability (Sandercock et al., 2001; Sandercock and Mitchell, 2003). These changes in sarcolemma integrity and permeability are thought to play a key role in the deleterious effects of heat stress on intracellular water loss and WHC.

The effects of heat stress on the muscle tissue and ultimately WHC are likely related to oxidative stress. Heat stress increases the production of reactive oxygen species (ROS) (Feng et al., 2008; Mujahid et al., 2005, 2006, 2007a, 2007b) and induces lipid peroxidation (Feng et al., 2008; Wang et al., 2009; Mujahid et al., 2009) within skeletal muscle tissue. Heat stress and oxidative stress decrease Ca^{2+}-ATPase activity and disrupt intracellular Ca^{2+}-homeostasis within muscle (Feng et al., 2008). Thus, increased intracellular calcium and free radical production in muscle are thought to influence WHC in poultry by inducing metabolic dysfunction, increasing the rate of postmortem muscle glycolysis, and disrupting cell membrane integrity.

Oxidative stress and protein oxidation may also directly impair the postmortem ability of muscle proteins to bind water. It is known that oxidation influences muscle protein functionality during processing and storage (Ooizumi and Xiong, 2004). Heat stress prior to slaughter has been shown to

increase the oxidation of both sarcoplasmic and myofibrillar proteins in broiler breast muscles (Wang et al., 2009). In this same study, muscle protein oxidation due to heat stress was accompanied by significant reductions in protein solubility and WHC.

In cooler climates and seasons, WHC characteristics of fresh poultry meat can also be influenced by cold stress on the live birds. The exposure of broilers to extremely low temperatures ($\leq 0^{\circ}C$) during transportation and lairage increases ultimate pH and improves WHC attributes important in both ground (salt-induced water uptake, processing cook yield) and intact (cook loss, thaw loss) breast meat (Dadgar et al., 2010, 2011, 2012a, 2012b). Even more moderate cold exposure ($0-20^{\circ}C$) during transportation and lairage has been shown to increase average pH and WHC in broiler breast meat compared to samples from birds experiencing $20-30^{\circ}C$ temperatures throughout preslaughter activities (Dadgar et al., 2010). Although cold exposure can reduce the incidence of PSE-like meat and result in breast meat with greater water binding capabilities that may be beneficial in further processed products, cold stress on birds increases the incidence of dark, firm, dry (DFD) meat and extreme color variations in breast meat (Dadgar et al., 2010, 2011, 2012a, 2012b).

4.10 POSTMORTEM FACTORS

The slaughter and postmortem processing of poultry induces biochemical and structural changes within the muscle tissue that influence WHC attributes in the final product. The first processing stages of typical commercial poultry slaughter include: shackling, immobilization (stunning, exsanguination), feather removal (scalding, picking), evisceration, and carcass chilling. To facilitate earlier deboning times, some commercial slaughtering facilities also utilize carcass electrical stimulation (ES) immediately after slaughter. During second processing, chilled carcasses can be packaged for marketing whole or cut into parts, deboned, or further-processed depending upon the desired final product. With regard to their specific effects on WHC, the processing steps of stunning, ES, chilling, and deboning time have been more thoroughly investigated than other stages of first and second processing. Variations in these particular steps are more likely to influence WHC due to their impact on muscle activity, energy metabolism, temperature decline, and rigor development. Further-processing steps and the addition of ingredients are also known to impact poultry meat functionality and WHC but are beyond the scope of this chapter.

4.10.1 Stunning

The process of stunning birds to render them unconscious prior to slaughter is known to influence overall carcass quality and the onset of rigor mortis,

but its specific effects on WHC have not been fully elucidated (Fletcher, 2002; Joseph et al., 2013). The effects of stunning on meat quality are dependent upon the type and parameters of the stunning system. Most commercial slaughter plants utilize either low-voltage electrical stunning (most common in the United States) or high-current electrical stunning (used in Europe) via an electrified water bath. Gas stunning systems and low-atmosphere pressure stunning (LAPS) systems are also commercially available and utilized, but to a much lesser extent than electrical stunning.

Electrical stunning slows down early postmortem pH decline and delays the onset of rigor mortis by approximately 4–6 h in poultry carcasses but does not influence ultimate pH or cook loss in breast meat (Lee et al., 1979; Kim et al., 1988; Papinaho and Fletcher, 1995, 1996; Alvarado and Sams, 2000b). Differences in early postmortem muscle metabolism and rigor development due to electrical stunning are thought to be related to the degree of perimortem muscle activity that occurs within the birds (Papinaho et al., 1995). It has been observed that electrical stunning at 50 V may cause a decrease in broiler breast meat pH at 2 h postmortem and an increase in drip loss (Huang et al., 2014). However, the voltage level of electrical stunning (15, 50, or 100 V) in broilers does not seem to influence ultimate pH, cook loss, or filter press moisture loss in the breast meat (Huang et al., 2014). Low-field NMR data has suggested that mid-voltage electrical stunning may cause a shift in water from the intramyofibrillar to the extramyofibrillar compartments within the tissue, thus reducing WHC (Huang et al., 2014). With electrical stunning of turkeys, increasing the current frequency (50–600 Hz) has been shown to accelerate pH decline in breast meat from 0 to 5 h postmortem and to increase cook loss but not drip loss (Sante et al., 2000).

Although gas stunning of birds may cause a slightly more rapid pH decline early postmortem than electrical stunning (Raj et al., 1990; Poole and Fletcher, 1998), WHC attributes such as cook loss and expressible moisture are not different between electrically stunned and gas stunned broilers (Raj et al., 1990; Poole and Fletcher, 1998; Kang and Sams, 1999; Savenije et al., 2002b) and turkeys (Northcutt et al., 1998). Compared to electrical stunning, LAPS accelerates pH decline in broiler breast meat from 0 to 4 h postmortem but does not influence ultimate pH or cook loss (Battula et al., 2008; Schilling et al., 2012). In marinated breast fillets, LAPS does not affect brine uptake but increases cooking loss (Schilling et al., 2012). Although differences in the types and operating parameters of stunning systems may influence early postmortem pH decline and rigor onset, ultimate meat pH and WHC attributes do not seem to be drastically impacted by variations in the stunning practices commonly used throughout the poultry industry.

4.10.2 Electrical Stimulation

Postmortem ES of carcasses immediately following slaughter is a method that is increasingly being used commercially to prevent toughness problems

associated with early deboning. The muscle contractions caused by ES accelerate ATP and glycogen depletion within the muscle and hasten the onset of rigor mortis. As a result of ES increasing the rate of early postmortem pH decline while muscle temperatures are still high, it has been speculated that ES of carcasses may potentially cause additional postmortem protein denaturation and diminish WHC. Although the effects of ES on meat tenderness are well-established (Sams, 2002), data on the influence of ES on WHC in poultry have been inconsistent. Some studies observed that ES decreased cook loss and improved WHC in broiler breast fillets (Alvarado and Sams, 2000a; Dickens et al., 2002; Young et al., 2005; Perlo et al., 2012), while others reported that ES increased cook loss and expressible moisture loss (Froning and Uittenboogaart, 1988; Lyon et al., 1989, 2002; Young and Lyon, 1997). Numerous other studies, however, have not observed a consistent positive or negative effect of postmortem ES on WHC attributes such as cook loss, expressible moisture loss, or thaw loss in breast meat from broilers (Dickens and Lyon, 1995; Zocchi and Sams, 1999; Young and Buhr, 2000; Zhuang et al., 2010), turkeys (Maki and Froning, 1987; Janky and Birkhold, 1989; Owens and Sams, 1997), or ducks (Alvarado and Sams, 2000a). One report showed that when ES was followed by rapid carcass chilling there was no impact on cook loss or expressible moisture in broiler breast meat, but observed that ES decreased WHC if it was not followed by proper chilling (Castaneda et al., 2005). The divergent findings regarding the effects of postmortem ES on WHC attributes in poultry are likely due to varying ES operating parameters, chilling regimes, and deboning times between studies.

4.10.3 Carcass Chilling

Following evisceration, poultry carcasses are typically chilled to a temperature of 4°C or less within several hours. While carcass chilling is carried out to improve food safety and shelf-life by reducing microbial growth, chilling strongly influences WHC. Improper carcass chilling can result in elevated muscle temperatures which in combination with a rapid postmortem pH decline can result in poor WHC and an increased incidence of PSE-like meat. Even carcasses with a normal pH decline may develop PSE-like meat if improperly chilled. Rapid chilling of poultry carcasses decreases the degree of postmortem protein denaturation that occurs within the muscle and reduces the potential for developing inferior WHC attributes. Improper chilling is typically more problematic in turkey carcasses than broiler carcasses due to overall size and muscle mass.

Slower carcass chilling rates and elevated postmortem muscle temperatures (>30°C) have been shown to negatively influence WHC attributes in both turkey (McKee and Sams, 1998; Alvarado and Sams, 2002; Alvarado and Sams, 2004; Zhu et al., 2013) and broiler (Zhu et al., 2011) breast meat due to accelerated postmortem glycolysis and rigor mortis. Delayed carcass chilling has also been shown to decrease ultimate muscle pH, protein

extractability, and cook yield in turkey breast meat (Rathgeber et al., 1999a). In hot-boned prerigor turkey breast and thigh muscles, delayed chilling and elevated postmortem temperatures were found to increase drip loss (Lesiak et al., 1996, 1997). Using a research model to investigate PSE-like meat, it was observed that maintaining hot-boned prerigor turkey breast muscle at elevated temperatures (40°C) for up to 6 h postmortem prior to chilling causes excessive whole muscle chilling loss, drip loss, thaw loss, and cook loss (Molette et al., 2006).

Poultry carcasses are commonly chilled by either water immersion chilling or air chilling. During immersion chilling, which typically lasts 45−110 min depending on carcass size, water pickup from the chill tank typically increases carcass weights by 4%−8% (James et al., 2006). This moisture pickup has little to do with the WHC of the muscle. Most of the moisture is absorbed into the skin or trapped in pockets under the skin and is subsequently lost during cut up, storage, or further processing (Klose et al., 1960). With air chilling, carcasses are typically kept on shackles and passed through rooms of circulating cold air (−8°C to 2°C) for 1−3 h. Water misting and relative humidity control are typically used to enhance cooling and prevent moisture losses that can occur in carcasses during air chilling. The total moisture content of the muscle tissue does not seem to be influenced by the carcass chilling method (Jeong et al., 2011a, 2011b; Zhuang et al., 2008; Bowker et al., 2014). While a few studies have reported slightly lower drip loss (Jeong et al., 2011b; Bowker et al., 2014b; Zhuang et al., 2013) and cook loss (Zhuang et al., 2013; Huezo et al., 2007a, 2007b) in broiler breast meat from air-chilled carcasses, other studies have not observed a difference in WHC attributes between air- and immersion-chilled carcasses (Jeong et al., 2011a; Zhuang et al., 2008; Permulla et al., 2011). It has also been shown that air-chilling broiler carcasses may slightly improve brine pick-up and retention in marinated breast fillets compared to immersion chilling (Carroll and Alvarado, 2008). Thus, while the postmortem temperature profile of the muscle plays a vital role in determining WHC, if done properly the specific method used for rapidly chilling poultry carcasses probably only accounts for minor variations in fresh poultry WHC.

The negative influence of improper carcass chilling on WHC attributes is the result of high muscle temperatures accelerating the rate of early postmortem pH decline and rigor mortis development. In both turkey and broiler breast meat, elevated postmortem muscle temperatures (40°C) cause a more rapid decrease in pH and increase in R-value (inosine:adenosine ratio) from 0 to 4 h postmortem (Zhu et al., 2011, 2013; McKee and Sams, 1998). Maintaining deboned breast meat at elevated temperatures, however, does not seem to impact ultimate muscle pH and the extent of postmortem glycolysis (Zhu et al., 2011, 2013). Thus, elevated muscle temperatures seem to primarily increase the rate of early postmortem glycolysis and rigor development. These in turn reduce WHC by increasing both sarcoplasmic and

myofibrillar protein denaturation in turkey breast meat (Zhu et al., 2013) and sarcoplasmic protein denaturation in broiler breast meat (Zhu et al., 2011). Data collected in turkey breast meat suggest that high carcass temperatures early postmortem may cause rapid muscle glycolysis by activating AMP-activated protein kinase (AMPK). AMPK plays a role in cellular energy homeostasis in living muscle fibers and has been shown to be a key enzyme controlling early postmortem pH decline in pork (Shen et al., 2006). However, the role that AMPK and the activation pathway of the glycolytic enzymes plays in the development of poor WHC attributes in poultry meat is not fully understood.

4.10.4 Deboning

In order to meet growing market demands for boneless, skinless breast fillets and further-processed products, carcasses are often deboned postchilling. Processors are increasingly deboning carcasses immediately postchill in order to improve production efficiency and save energy. The timing of deboning in relation to rigor mortis development strongly influences meat quality. If the breast muscle is deboned prior to the completion of rigor mortis, muscle fibers can subsequently shorten during rigor development due to the lack of skeletal restraints causing less tender meat. To remove breast muscles from carcasses without excessive meat toughening, 4–6 h of aging prior to deboning is recommended for broilers to allow for complete rigor development. Although postmortem deboning time is known to strongly influence meat tenderness, WHC attributes may also be affected, but to a lesser degree. In general, the cook yields of breast meat have been observed to increase with the duration of postchill aging prior to deboning in both broilers (Dickens and Lyon, 1995; Huezo et al., 2007a; Northcutt et al., 2001; Liu et al., 2004; Brewer et al., 2012; Bowker et al., 2016) and turkeys (Obanor et al., 2005). Increased drip loss has been observed in early-deboned (2 h postmortem) broiler breast meat (Bowker et al., 2016). Other studies, however, have not observed a consistent deboning time effect on WHC in chicken breast meat (Brewer et al., 2012; Lyon et al., 1992; Souza et al., 2005; Mehaffey et al., 2006). Diminished WHC in early deboned breast meat may be related to a reduction in the intra-myofibrillar space available for holding water caused by the sarcomere shortening associated with prerigor deboning (Papa and Lyon, 1989).

4.10.5 Postmortem Time and Storage

Due to intrinsic biochemical and structural changes within muscle tissue, various WHC attributes of fresh poultry meat have been observed to change throughout postmortem processing and refrigerated storage. Postmortem time seems to differentially influence the various measures of WHC. Numerous

studies showing increasing drip loss accumulation with postmortem time indicate that exudate moisture is continually expressed from the muscle tissue during the first 24 h postmortem and throughout extended postmortem storage (6−9 days) in both broiler and turkey meat (Molette et al., 2003; Bowker and Zhuang, 2013, 2015; Lee et al., 2009). Most of the fluid loss from meat with postdeboning aging seems to occur early postmortem. In hot-boned turkey breast meat it has been shown that the majority of the drip (i.e., exudate) that is lost from the meat during the first 24 h postmortem occurs during the first 3 h postmortem (Molette et al., 2003). In chicken breast meat deboned between 1.5 and 6 h postmortem, it has been observed that approximately 50% of the total drip that accumulates over 6−7 days of refrigerated storage occurs within the first 2 days postmortem (Bowker and Zhuang, 2015; Lee et al., 2009). Using the filter press method for determining WHC, however, it was observed that the amount of expressible fluid loss was similar at 2 h, 24 h, and 7 days postmortem in early-deboned broiler breast fillets (Zhuang and Savage, 2012).

Postdeboning aging time seems to have a variable influence on measures of WHC such as cook loss and salt-induced water uptake. Cook loss has been observed to gradually increase from 0 to 168 h postmortem in hot-deboned broiler breast fillets (Tomaszewska-Gras et al., 2011). In broiler breast fillets deboned at 1.5−6 h postmortem, it has been reported that cook loss tends to slightly increase (Lee et al., 2009; Dunn et al., 1993) or to remain unchanged (Zhuang and Savage, 2012; Bowker et al., 2016) with extended postdeboning aging for 6−7 days. In broiler leg muscles, however, cook loss was found to increase from 0 to 6 h, decrease from 6 to 24 h, and then remain steady from day 1 to day 7 postmortem (Tomaszewska-Gras et al., 2011). Using the ability of muscle to pick up and retain added salt water as an indicator of WHC, it has been observed that WHC in hot-boned broiler breast meat increases from 0.5 to 2 h postmortem, decreases from 2 to 8 h postmortem, and then gradually increases again from 8 to 24 h postmortem (Bowker and Zhuang, 2014). With commercially processed pale- and dark-colored fillets, salt-induced water uptake was found to be greater at 24 h postmortem than at 6 h postmortem (Bowker and Zhuang, 2015). It has also been observed that salt-induced water uptake in broiler breast meat at 24 h postmortem is greater than at 2 h postmortem, but that WHC as measured by this method does not change with additional postmortem storage for 7 days (Zhuang and Savage, 2012).

Comprehensive studies on the evolution of WHC throughout postmortem processing and storage of fresh poultry meat are lacking; thus the mechanisms controlling WHC changes are not fully understood. Available evidence suggests that WHC changes during the early postmortem period (0−6 h) are likely related to changes in pH and rigor development whereas WHC changes at later postmortem times and throughout extended storage may be related to postmortem protein degradation. Interpreting the available data on

the relationship between WHC and postmortem time is complicated by study to study variations in WHC measurement techniques, deboning time, and storage conditions.

Poultry meat is often stored frozen prior to either further processing or usage by the consumer. Freezing and thawing of poultry causes fluid to be lost from the muscle tissue and a reduction in WHC (Zhuang and Savage, 2012; Yoon, 2002; Lee et al., 2008a). Both the parameters of the freeze/thaw cycle (temperature, rate of freeze/thawing, duration of storage) and characteristics of the poultry meat itself (debone time, postdeboning aging time, initial quality, etc.) influence the effects on WHC. With long-term frozen storage of broiler breast meat from 0 to 8 months, there is an increase in thaw loss and cook loss and a decrease in cooked meat moisture content with storage time (Lee et al., 2008a). Short-term frozen storage and subsequent thawing of breast meat are thought to increase in the proportion of water held in the extracellular compartments of the muscle fibers (Zhuang and Savage, 2012). The reduction in WHC with freezing/thawing is due to ice crystal formation disrupting the cell membrane integrity and muscle structure (Yoon, 2002; James, 2002), myofibrillar shrinkage (Yoon, 2002; Van Laack, 1999), and protein denaturation (Chan et al., 2011).

4.11 SUMMARY

The ability of fresh poultry meat to retain inherent or added moisture is a highly variable and complex meat quality attribute. Numerous interacting antemortem and postmortem factors can influence WHC characteristics in the final meat product. Biochemical and structural changes within the muscle tissue during the conversion of muscle to meat greatly impact WHC. The distribution of water between the various compartments within the muscle (intramyofibrillar, extramyofibrillar, extracellular) throughout slaughter, processing, and storage plays a key role in WHC. Although much is known about how various factors influence WHC, a comprehensive understanding of the underlying mechanisms controlling WHC in fresh poultry meat is still lacking.

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Chapter 5

Methods for Measuring Meat Texture

Amit Morey[1] and Casey M. Owens[2]
[1]Auburn University, Auburn, AL, United States, [2]University of Arkansas, Fayetteville, AR, United States

5.1 MEAT TENDERNESS

Boneless, skinless broiler breast meat is one of the most consumed meat products in the United States and around the world, especially with rising household incomes. It has many positive attributes that make it popular with consumers. It is low in fat, has a bland flavor profile making it versatile in food dishes, and is reasonably priced. It is also available in a variety of forms. Broilers are typically raised to various market ages in order to produce birds of various weights depending on the intended markets (e.g., whole bird, fast food, and further-processing markets).

Tenderness of broiler breast meat is one of the most important attributes for consumers. Broilers have traditionally been considered an inherently tender product; however, there are factors that can negatively impact the tenderness of broiler breast meat including deboning time, age of the broiler, and moisture of the product. In the past couple of decades, some changes have been made in the production and processing of broilers that have made these factors more or less important. For example, postmortem aging times have shortened by harvesting breast meat earlier and bird size/age has increased in recent years. Most of the broilers processed today go through some sort of further processing which can be as simple as portioning and marination, or as complex as producing deli meats or chopped and formed products. The whole muscle products are particularly susceptible to tenderness/toughness issues. These types of products are also generally the premium products so consumer acceptance of these products becomes even more important.

As the poultry industry developed, one of the simpler forms of adding value was to debone breast meat. Initially, this deboning process was commonly performed anywhere from 8 to 24 h postmortem to allow for meat to age on the bone for a period of time. This aging period allowed for rigor mortis

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completion (a metabolic process) and the results were a tender product. However, aging carcasses prior to deboning was (and is) expensive due to costs associated with refrigeration, labor, and lost yield. Therefore, in the past couple of decades, aging periods have shortened considerably to allow increased throughput of the product in order to meet consumer demands for breast meat, as well as to obtain increased yields associated with early deboned meat. Many processors are deboning breast meat right out of the chiller which is as early as 2 h postmortem in efforts to streamline their process. While there are economic benefits associated with reduced aging, these shortened aging periods do not always allow rigor completion prior to deboning. When deboning in a prerigor state (<4–6 h postmortem), muscle can contract because of the sufficient energy supplies that remain in the muscle when the stimulus (cutting action) is applied. This is a form of induced sarcomere shortening where the sarcomeres, or basic contractile units of muscle, shorten during contraction (Fig. 5.1) as a result of early deboning. This results in a denser muscle, due to more myofibrillar overlap and, therefore, tougher meat when cooked. Because of this toughening effect, it has been recommended that broiler carcasses be aged for at least 4–6 h prior to deboning to allow for energy to be depleted sufficiently to prevent induced sarcomere shortening. It is well documented that tenderness (as measured by shearing) improves (decreasing shear values; Fig. 5.2) as the aging time prior to deboning increases (Dawson et al., 1987; Cavitt et al., 2005). Furthermore, the variation of tenderness among fillets is much greater when fillets are deboned early postmortem so that some fillets may be much tougher than others and this variation decreases as aging time increases (Cavitt et al., 2005). In addition to debone time, or sarcomere shortening, age of the bird can also influence meat tenderness. Older birds tend to be slightly tougher than younger birds which may be due to changes in collagen solubility and/or muscle fiber properties such as fiber diameter. Though there is a tradeoff for processors in terms of economics and quality, there are some other means to provide a tender product even when deboning early including electrical stimulation and marination (Table 5.1).

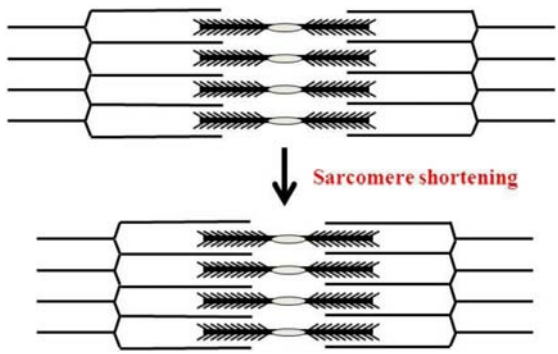


FIGURE 5.1 Sarcomere shortening due to early deboning.

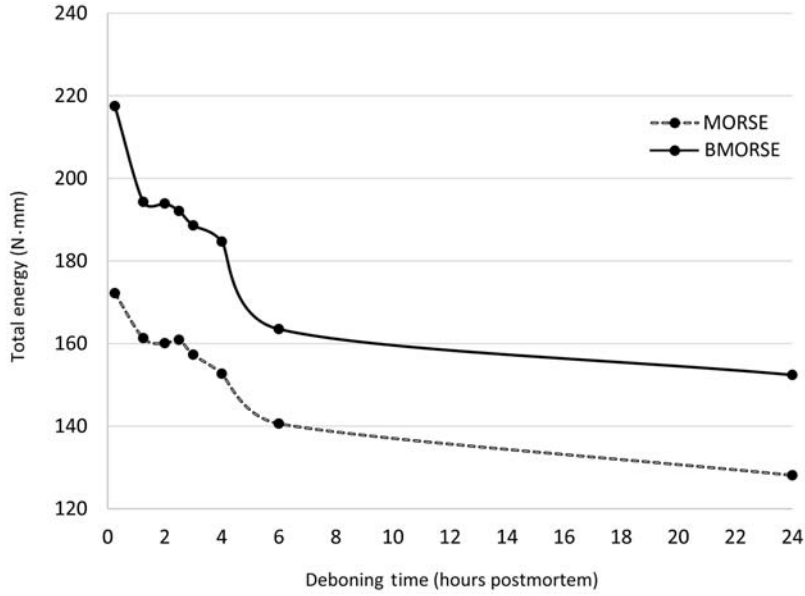


FIGURE 5.2 Relationship between MORSE and BMORSE ($R^2 = 0.99$) for broiler breast meat sheared various times postmortem. *Adapted from Lee, Y.S., Owens, C.M., Meullenet, J.F. The Meullenet–Owens Razor Shear (MORS) for predicting poultry meat tenderness: its applications and optimization. J. Text. Stud., 39(6) (2008) 655–672.*

There is a need for a quality control program for eating-quality characteristics, specifically regarding the tenderness of broiler breast meat. There are many factors, as previously discussed, that can play a role in the variation of product quality that consumers can encounter. Once consumers have a bad experience with a certain product, they are less likely to purchase the product and/or brand again for some time. This can definitely affect product loyalty. Therefore, implementing a quality-control program can allow processors to assess quality factors, specifically tenderness-related attributes. There are several methods that are proven for use in assessing meat tenderness using instrumental or sensory methods. The following discussion will focus on typical methods used in industry and research to assess meat tenderness. While the discussion is primarily focused on tenderness assessment of poultry breast meat, some reference information is included on the texture assessment of dark meat (Table 5.2).

5.1.1 Warner–Bratzler Shear Force Analysis

Warner–Bratzler shear force (WB) is probably the oldest and the most used method to detect the texture of meat, poultry, and seafood (García-Segovia et al., 2014). The WB method works on the principle of compression, tensile,

TABLE 5.1 Classification of Acceptance and Intensity of Tenderness for Warner–Bratzler Shear, Allo–Kramer Shear, and Meullenet–Owens Razor Shear

Attribute	Hedonic Scale		Warner–Bratzler Shear Force (kgf)	Allo–Kramer Shear Value (kgf/g)	Meullenet–Owens Razor Shear Force (N)	Meullenet–Owens Razor Shear Energy (N · mm)
Tenderness acceptance	Dislike extremely	1	≥17.9	≥18.6	>19.03	≥236.98
	Dislike very much	2	15.7–17.9	16.5–18.5	17.6–19.0	218.3–237.0
	Dislike moderately	3	13.5–15.7	14.4–16.5	16.1–17.6	199.6–218.3
	Dislike slightly	4	11.3–13.5	12.3–14.4	14.7–16.1	181.0–200.0
	Neither dislike nor like	5	9.1–11.3	10.2–12.3	13.2–14.7	162.3–181.0
	Like slightly	6	6.9–9.1	8.1–10.2	11.8–13.2	143.6–162.3
	Like moderately	7	4.7–6.9	6.0–8.1	10.3–11.8	124.9–143.6
	Like very much	8	2.5–4.7	3.9–6.0	8.9–10.3	106.3–124.9
	Like extremely	9	≤2.5	≤3.9	≤8.9	≤106.3
Tenderness intensity	Extremely tough	1	≥16.0	≥16.7	≥18.00	≥222.0
	Very tough	2	14.1–16.0	14.9–16.7	16.7–18.0	205.5–222.0
	Moderately tough	3	12.2–14.1	13.1–14.9	15.4–16.7	189.0–205.5
	Slightly tough	4	10.3–12.2	11.3–13.1	14.1–15.4	172.5–189.0
	Neither touch nor tender	5	8.4–10.3	9.4–11.3	12.8–14.1	156.0–172.5
	Slightly tender	6	6.5–8.4	7.6–9.4	11.5–12.8	139.5–156.0
	Moderately tender	7	4.5–6.5	5.8–7.6	10.2–11.5	123.0–139.5
	Very tender	8	2.6–4.5	4.0–5.8	8.8–10.2	106.5–123.0
	Extremely tender	9	≤2.6	≤4.0	≤8.8	≤106.5

Modified from Cavitt, L.C., Meullenet, J.F., Gandhapuneni, R.K., Youm, G.W., Owens, C.M. Rigor development and meat quality of large and small broilers and the use of Allo-Kramer shear, needle puncture, and razor blade shear to measure texture. Poult. Sci. 84(1) (2005) 113–118.

TABLE 5.2 Texture Analysis of Dark Meat From Various Strains of Broilers

Chicken Strain	Sample Type	Texture Measurement Method	Texture	Source
Commercial broiler	Whole muscle	Warner–Bratzler shear (N)	Prerigor: 75.27 N Postrigor: 37.50 N	Yu et al. (2005)
New Hampshire dual-purpose breed	Whole muscle	Warner–Bratzler shear (N)	New Hampshire breed: 23.82 N	Karlsson et al. (2009)
Commercial broiler			Commercial Broiler: 23.08 N	
Unspecified	Whole muscle	Sensory analysis (15-point unstructured scale: 0—low intensity and 15—high intensity)	Day 2 Average Sensory Scores (irrespective of treatments) represented Tenderness: 10.35–10.74 Firmness: 6.40–7.28 Fibrous: 2.73–3.72	Jongberg et al. (2014)
Korean native chicken	Ground meat patty	Texture profile analysis using a needle probe	Raw thigh meat: Hardness: 0.31 (kg) Springiness: 0.96 mm Cohesiveness: 0.44% Gumminess: 0.13 kg Chewiness: 0.13 kg	Jung et al. (2011)

(*Continued*)

TABLE 5.2 (Continued)

Chicken Strain	Sample Type	Texture Measurement Method	Texture	Source
			Cooked thigh meat: Hardness: 4.81 kg Springiness: 0.84 mm Cohesiveness: 0.42% Gumminess: 2.02 kg Chewiness: 1.69 kg	
Organic, free-range, and conventional broilers	Whole muscle	Shear force using star probe	Conventional: 1.45 kg/cm^2 Organic: 1.88 kg/cm^2 Free-range: 1.89 kg/cm^2	Husak et al. (2008)
Thai indigenous chicken	Whole muscle	Warner–Bratzler shear	Raw: 5.20 kg Cooked: 4.67 kg	Wattanachant et al. (2004)

and shear forces on muscle fibers (García-Segovia et al., 2014). WB attachment to a texture analyzer consists of a single blade with a V-shaped notch which shears the meat sample perpendicular to the muscle fiber direction and the maximum shear force is reported as N or Kg (Fig. 5.3). The force-deformation curves obtained through WB analysis have two peaks, the first peak corresponds to changes in myofibrillar protein and the second to connective tissue component (Møller, 1981).

Some of the most important factors affecting variability, accuracy, and repeatability of WB testing include:

1. *Thawing temperatures*: Wheeler et al. (1996) found that thawing steak at various temperatures from −2°C to 12°C had a significant impact on the WB measurements.
2. *Cooking temperatures*: Møller (1981) demonstrated that cooking temperature (60°C or 80°C) affected the myofibrillar and connective force-deformation thus ultimately affecting result interpretation and correlation with sensory characteristics.



FIGURE 5.3 The V-shaped blade used to determine Warner−Bratzler shear force.

3. *Orientation of muscle fiber*: fiber orientation should be parallel to the core and not perpendicular such that the blades can cut across the muscle fiber bundle (Wheeler et al., 1996).
4. *Muscle type and location*: Belew et al. (2003) found WB textural differences among 40 bovine muscle groups as well as between various locations within the same muscle.

The WB method has been traditionally used for beef, pork, and other mammalian species. It has also been used with poultry though the methodology is slightly modified. With poultry, a strip of meat (1.9 cm wide) cut parallel to the muscle fibers is used rather than a core of meat (Lyon and Lyon, 1991). The method can be performed with a portable WB device or more commonly for poultry, with a material testing machine such as an Instron Universal Testing System or Texture Analyser (Stable Micro Systems Ltd). These material testing devices are also commonly used for the methods discussed in the rest of this chapter.

5.1.2 Allo–Kramer Shear Force Analysis

The Allo–Kramer shear (AK) cell was introduced in the early 1950s as a mechanical method to objectively measure texture. The metallic shear cell comprises of two parts; blades and the base (Fig. 5.4). The blunt blades (5–13) of equal length are arranged in parallel at a fixed distance and correspond to the slots on the base. In a simple mechanism, a sample is placed on the base, approximately in the center, the blunt blades compress the sample, shear through it and pass through the slots. The software attached to the machine detects the peak shear force (kg/g) required to go through the meat. Force measured in Newtons can also be obtained and has been more commonly used for scientific reporting in recent years.

In addition to the blades and the base, sample preparation play a significant role in texture analysis via AK. Researchers have found that meat samples of varying sizes and dimensions can have an impact on the shear force (DeMan and Kamel, 1981) and it can be suggested that a standard sample size can reduce variability and increase ease of comparison between studies. Poultry researchers have used a set sample weight approximately 5–7 g and dimensions approximately (L 4 cm × W 2 cm × D 0.5 cm) (Owens et al., 2004). In addition to the size and shape, samples can also be cut from a specific region on the meat, e.g., the crown region of the chicken breast fillet.

The AK method has been used extensively in studying meat quality. Lyon and Lyon (1991) found that the AK values had a significant correlation ($R^2 = 0.86$) with the sensory texture values of chicken breast meat deboned at various times. While Papinaho and Fletcher (1996) used AK to optimize electrical stunning parameters and debone times such that the meat texture is not impacted. They also reported that high amperage stunning (125 mA) can



FIGURE 5.4 Allo–Kramer shear cell.

lead to tougher meat upto 10 h postmortem. Some other researchers used AK to correlate meat texture with color with varying success. Although Young et al. (1996) and Barbut (1993) demonstrated that there was a correlation between poultry meat color and texture, Allen et al. (1998) found no significant correlation between these parameters.

The AK test has been highly effective in detecting meat texture until the recent past. However, changes in the strains of food animals, nutrition, growth rate, and other changing animal husbandry factors are causing changes in the muscle structure and composition. The best example is the rapid adoption of fast-growing big-bird strains in the poultry industry wherein chickens are grown up to 8–10 lbs compared to 4–6 lbs in the recent past. This alteration has led to the development of myopathies such as white striping and woody breast (Bauermeister et al., 2009; Bilgili, 2013). These myopathies are causing textural issues in meat however they are not clearly detected by AK. Mazzoni et al. (2015) demonstrated that although chicken breast meat exhibited significant myodegeneration via histopathological analysis, cook loss, as well as difference in proximate composition, there was not a significant difference in the

AK values between the samples. Their study indicated that AK might not be the best method to detect meat quality with myodegeneration. Similarly, Schrader et al. (2016) demonstrated that AK values of chicken breast meat with mild, moderate, and severe woody breast myopathy were not effective in detecting woody breast. The authors indicated that novel methods must be used to study meat quality in modern big chicken strains.

5.1.3 Texture Profile Analysis

Meat texture is a multidimensional attribute that includes several characteristics such as chewiness, adhesion, springiness, among others that cannot be measured by shear force analysis. To study the characteristics of meat other than shear force, a compression method called Texture Profile Analysis (TPA) was proposed by Friedman et al. (1963) and Szczesniak et al. (1963) which reflects mastication of food. The TPA apparatus used for meat and meat-product analysis usually consists of a flat circular disc/probe attached to the compression arm of the texture analyzer (Fig. 5.5). It is recommended



FIGURE 5.5 Texture profile analysis probe attached to a texture analyzer.

that the meat sample should be of a smaller diameter compared to the probe. The software operating the compression arm compresses the sample, retreats to let the sample bounce back/relax followed by second compression to mimic "two bites." Based on the "two bites" compression data, several textural attributes can be determined such as hardness, springiness, chewiness, gumminess, cohesiveness, fracturability, and resilience.

Onega et al. (2001) and de Huidobro et al. (2005) demonstrated that TPA analysis is a better predictor of meat texture compared to WB because of: (1) better understanding of meat texture attributes such as hardness, springiness, and chewiness, (2) significant correlation with sensory data, and (3) higher accuracy (low coefficient of variation). Similarly, Caine et al. (2003) indicated that TPA parameters, such as hardness, and adhesiveness, are useful predictors of textural properties of rib steaks and could help in explaining the variation in sensory panel perception of beef tenderness compared to WB analysis. In addition to whole muscle, TPA is routinely used to determine texture of formed and emulsified products such as deli and hot dogs, respectively (Luckett et al., 2014; Morey et al., 2012). Based on the above-stated reports and other studies, it must be noted that TPA parameters describing texture may vary depending on the product being tested. Researchers should conduct statistical analysis such as step-wise regression to determine the parameters that can best explain variability in texture of meat. However, data can be transformed, e.g., by logarithmic transformation, to improve correlation between sensory attributes and corresponding TPA parameters (Meullenet et al., 1998).

TPA is a highly sophisticated method that can calculate several parameters using the software. It is very easy for TPA users to report all the parameters although they might be irrelevant, report wrong parameters, or misinterpret the data. Since the TPA test is a "two-bite" test, it is imperative that the product does not crack/break in the first compression or the user will not be able to get appropriate data from the second compression. Investigators should understand the nature and the composition of the product to determine the compression force and deformation, e.g., between hot dogs and cheese. Moreover, the compression force should be ideally decided on the bite force exerted by the target consumer while masticating (e.g., the bite force required by an elderly population will be different compared to youth) (Trinh and Glasgow, 2012). Most researchers use 75% deformation with parallel plates with a crosshead speed of 2 mm/s (Rosenthal, 2010).

5.1.4 Meullenet–Owens Razor Shear (MORS) and Blunt MORS

Common texture analysis methods such as AK, WB, and TPA can be subject to variation due to excising the sample from the whole muscle or variation in texture due to location (Owens and Meullenet, 2010). Hence there was a

need to develop novel methods that can be used on whole samples thus reducing labor, time for analysis, and variability due to location.

Cavitt et al. (2001) were the first to report the application of "razor blade shear" as a method for texture measurement later renamed as the Meullenet–Owens razor shear (MORS). They reported that the razor blade (RB) method was able to detect differences in the texture of cooked chicken breast meat similar to the AK method (Cavitt et al., 2005). Moreover, the RB method provided an additional texture parameter "shear energy" along with traditional "shear force" measurement thus adding a new dimension to meat texture analysis. Some of the major advantages of the MORS method are: (1) it reduces experimental error due to sample preparation, (2) the method is independent of sample dimensions, and (3) there is no sample preparation time (Lee et al., 2008).

MORS attachment to the texture analyzer consists of a metal stem to hold a RB (24 mm height × 8.9 mm width) which is set to penetrate 20 mm below the meat surface (Fig. 5.6 and Fig. 5.7). The cooked whole-meat



FIGURE 5.6 Meullenet–Owens razor shear device (blunt blade shown).



FIGURE 5.7 Razor blade (#17 blade, 8.9 mm width) used for Meullenet–Owens razor shear.

sample is placed on the platform such that the blade is perpendicular to the muscle fibers. As the blade shears through the meat, data is recorded via the software program and reported as shear force (N) and shear energy (N · mm). Analysis can be repeated at multiple spots on the same meat piece to account for the textural variation within the meat. It is important to replace the blade every 100 readings to ensure that sharpness does not affect the texture measurements.

The MORS method was further modified to use a blunt blade (BMORS; Fig. 5.6) instead of a sharp blade for texture analysis especially when testing tough meat (Lee et al., 2008). As the blunt blade forces its way into the meat, the computer measures the force required to go through each layer of meat giving us a much more detailed picture of the texture throughout the cross-section of meat. Lee et al. (2008) have also demonstrated that BMORS is highly correlated with MORS and can be used to predict meat tenderness. However, the BMORS force and shear energy correlate slightly higher with consumer tenderness acceptance and intensity scores compared to MORS, proving that BMORS might be a better method than MORS.

Several researchers have also used the MORS method to detect meat quality, such as in the effects of marination on chicken meat (Saha et al., 2009; Perumalla et al., 2011), and to study the effect of broiler strain, sex, and debone time on chicken meat texture (Brewer et al., 2012). Luckett et al. (2014) used BMORS in comparison with TPA and AK to study the texture of deli meats and found that BMORS is a reliable predictor of hardness and fibrousness compared to the other methods. The work of Sawyer et al. (2007) demonstrated that BMORS can be used to detect textural differences due to connective tissue among the various sections of beef semimembranosus. However, additional studies must be conducted on their application in beef and pork.

The MORS and BMORS methods have evolved at a time when the poultry industry is faced with complex meat texture due to woody breast myopathies. Chatterjee et al. (2016) reported that the MORS method was successful in detecting the textural differences among woody and normal chicken breast meat, however it was not able to detect differences between severe and moderately woody chicken breast meat in terms of shear force and energy. However, Sun et al. (2016) reported using peak counts from shear data to distinguish severe woody breast from normal using peak counts from both the MORS and BMORS methods. The number of peaks along the shear curve increases as severity of woody breast increases. This would be a way to assess the interior fibrous-like texture.

5.1.5 Sensory Evaluation

Szczesniak (2002) defined texture as "the sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the senses of vision, hearing, touch and kinesthetics." The sensory perception of texture is dependent on the mechanoreceptors in oral superficial structures (e.g., hard and soft palate, tongue, and gums), periodontal membrane at teeth roots and in muscles and tendons assisting in mastication (Guinard and Mazzucchelli, 1996). The tongue presses food against the palate to determine surface texture while the periodontal receptors determine textural response when chewing and also determine object thickness (Guinard and Mazzucchelli, 1996). The information received by the mechanoreceptors is used to adjust the chewing mechanism via altering muscular forces, chewing/biting direction, jaw movement, and chewing time before swallowing (Çakir et al., 2012). Although the mechanoreceptors play a significant role in determining the texture of food, the attitude toward texture varies greatly from person to person (Guinard and Mazzucchelli, 1996).

To determine and quantify sensory responses to meat texture, scientists have developed various descriptors that can correlate with instrumental texture analysis. In addition to the sensory attributes of hardness, springiness, chewiness, and gumminess, attributes such as initial juiciness, sustained juiciness, toothpacking, and denseness can be used to describe the texture of meat (AMSA, 2015; Liu et al., 2004; Maca et al., 1997). Fanatico et al. (2006) used the just-about-right (JAR) scale to determine the "appropriateness of the degree of tenderness" of whole chicken breast on the scale of 1−5 where 1 is much too tough, 2 is too tough, 3 is just about right, 4 is too tender, and 5 is much too tender. Several researchers have used the sensory spectrum method to evaluate predetermined meat texture attributes on a 15-point numerical intensity scale with 0 = none and 15 = extreme intensity (Lyon and Lyon, 2001).

It can be concluded that sensory texture perception, descriptors, and methods should be altered as per the target audience and the textural attributes of interest in particular meat or meat product.

5.2 COOKING METHOD

Cooking methods have varied in the literature for these instrumental and sensory analysis methods. For instrumental analysis, baking breast fillets on raised wire racks in foil-lined and covered pans is common (Sams, 1990). However, cooking in a cook-in bag (e.g., boilable bags heated in 85°C water; Lyon and Lyon, 1991) has also been used. Internal endpoint temperatures have varied with a minimum of 71°C up to 82°C, but a single endpoint temperature has been used within each study (Sams, 1990; Lyon and Lyon, 1991; Cavitt et al., 2005). Using an endpoint of 76°C or 78°C has been most common in recent years.

Cooking from a thawed or frozen state has also varied. In a recent study, Zhuang and Savage (2013) reported that cooking from a frozen state resulted in higher cook loss, shear values, and other sensory textural attributes which would indicate tougher meat when compared to cooking from a thawed state. Both methods have been acceptable in research, but the method used should be consistent within each study.

5.3 FUTURE NEEDS

Meat texture is a very important attribute and understanding it requires various instrumental and sensory methods. At present, the instruments used for texture detection often require samples to be pulled off the processing line for testing. It would be in the interest of researchers to collaborate with the industry to develop a rapid "on-line" method to detect meat texture such that "real-time" decisions can be made on meat quality. Moreover, the texture measurement methods must evolve to adapt to the changes in the genetics, feed efficiencies and growth rate of food animals. Methods must be developed to detect novel meat texture issues such as woody breast in fast-growing big-birds in the poultry industry. Such methods will help the poultry industry to sort out woody breast from normal meat and use it for other purposes.

In addition to instrumental meat texture analysis, researchers must explore the existing data published in literature to conduct meta-data analysis and even big data analytics to find out the hidden correlations that have not been observed in the past. For example, data analytics techniques can be used to determine new factors and correlations that affect the sensory and instrumental analysis as well as consumer acceptance.

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Chapter 6

Poultry Meat Color and Oxidation

Rafael Carvalho[1], Massami Shimokomaki[1] and Mario Estévez[2]
[1]State University of Londrina, Londrina, Brazil, [2]University of Extremadura, Cáceres, Spain

6.1 INTRODUCTION

Poultry production remains at the top of worldwide meat production (right after the pig industry) and the increasing demand for quality by consumers challenges the industry to produce safe, nutritive, appealing, and healthy poultry foods. Oxidative reactions occur throughout the entire food chain, from the living animal at the farm to the final consumer, and at all intermediate stages in between (Estévez, 2015). The genetic pressure toward fast growth rates and large breast muscles has made domestic birds particularly vulnerable to oxidative stress (Petracci et al., 2015; Kuttappan et al., 2016). The occurrence of oxidative damage in tissues leads to impaired biological functions and organic damage that may turn into health problems, compromised poultry performance, and altered meat quality (Fellenberg and Speisky, 2006). Of increasing concern are specific quality defects in poultry meat that have been associated with assorted causal factors, including impaired oxidative metabolism such as wooden breast and white striping (Estévez, 2015). Upon slaughter, the collapse of the endogenous antioxidant defences and the biochemical changes occurring in meat create a pro-oxidative environment that promotes subsequent oxidative reactions (Bekhit et al., 2013). Lipids, proteins, and other food constituents such as heme pigments are oxidized during processing, storage, and culinary preparation of poultry products, causing modifications in their sensory properties and nutritional value (Estévez, 2015). Poultry meat is recognized as highly sensitive to oxidative reactions owing to the high unsaturation degree of the muscle lipids (Min et al., 2008). The onset of rancidity and off-flavors in processed poultry products has been identified as a major concern of lipid oxidation (LOX) (Min and Ahn, 2005). Other studies have also documented the negative influence of protein oxidation (PROTOX) on the functionality, rheological properties and digestibility of muscle proteins (Lund et al., 2011;

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Estévez, 2011). The oxidation of pigments leads to color modifications with this attribute being critical for consumers when selecting poultry products at the market (Droval et al., 2012). Poultry meat color depends on myoglobin (Mb) concentration and chemical state, while it is affected by numerous factors such as bird age, gender, genetic background, diet, intramuscular fat, meat moisture content, preslaughter conditions, and processing variables (Totosaus et al., 2007). Color defects may appear by mechanisms in which oxidation may not have a direct implication such as bruises, altered postmortem glycogen metabolism, and assorted pathological conditions (Fletcher, 2002). As long as these chemical modifications are major threats to the quality of the poultry meat and processed poultry products, the application of antioxidant solutions seems to be unavoidable (Lund et al., 2011; Bekhit et al., 2013; Estévez, 2015). The growing industrial interest and a prolific scientific production have led to a large variety of antioxidant strategies with a high degree of innovation. In addition to the manipulation of animal feeds, the strategies involving muscle food formulation and innovative packaging solutions have gained considerable popularity (Gallo et al., 2012; Duthie et al., 2013; Bekhit et al., 2013; Delles et al., 2014; Falowo et al., 2014). The present chapter reviews the most recent advances in the field of color and oxidation in poultry and poultry products, emphasizing current concerns and future challenges in relation to consumer perception of these muscle foods.

6.2 POULTRY OXIDATION: BIOCHEMICAL BASIS

Whereas oxidation is typically linked to damage, spoilage, and degradation, oxidative reactions are known to be the basis for numerous biological functions including energetic metabolism (Kohen and Nyska, 2002). Oxidative stress appears as the outcome of the imbalance of pro-oxidants and the endogenous antioxidant defences in living tissues, involves an overproduction of reactive oxygen species (ROS), and leads to uncontrolled oxidative damage to cellular components (Cadenas and Davies, 2000; Kohen and Nyska, 2002). The main source of ROS in chicken muscles is the leakage of electrons from the respiratory chain in mitochondria during the reduction of molecular oxygen to water (Mujahid et al., 2007). Birds undergo oxidative stress as a result of numerous situations including heat stress, oxidized feeds, and assorted pathological conditions (Fellenberg and Speisky, 2006; Estévez, 2015).

After slaughter, poultry muscles undergo further oxidative reactions boosted by the collapse of endogenous antioxidant defences, the release of pro-oxidants and other biochemical changes occurring in postmortem muscle such as pH decline (Estévez, 2015). Some pro-oxidants are natural components of postmortem muscle such as transition metals (iron, zinc), Mb, and hydrogen peroxide. These chemical species induce LOX and PROTOX by promoting the formation of ROS through assorted mechanisms (Min and Ahn, 2005; Estévez, 2011; Soladoye et al., 2015). Besides ROS, reactive

nitrogen species (RNS) may also be able to induce oxidative stress in poultry muscle (Skibsted, 2011) and this is particularly applicable to cured (added nitrite) poultry foods. Nitrite is a precursor of peroxynitrite and other RNS that are responsible for the oxidation and nitration of muscle proteins. Reducing sugars, well-known for their implication in Maillard reactions, have also been recently found to play an active role as promoters of oxidative damage to meat proteins (protein glyco-oxidation) (Villaverde et al., 2014). Besides chemical agents, oxidative reactions can also be initiated in meat systems by physical agents such as γ-irradiation and light (photo-oxidation) (Soladoye et al., 2015) through their ability to generate ROS. Lázaro et al. (2014) studied the effect of UV irradiation on LOX and discoloration of chicken breast meat during 9 days of chilled storage.

Whereas both lipids and proteins are susceptible to ROS and RNS, the kinetics and chemistry behind lipid and PROTOX are fairly different. According to reaction constant rates between free radicals and biomolecules calculated by Cadenas and Davies (2000), the hydroxyl radical would react faster with particular proteins and amino acid residues such as methionine than with unsaturated fatty acids (Table 6.1). The early and preferential oxidation of these amino acids in biological systems is described as a

TABLE 6.1 Comparison of the Rate Constants for Reaction of Hydroxyl Radical Proteins, Amino Acids, and Other Relevant Biomolecules at pH c. 7

Substrate	Rate Constant ($dm^3\ mol^{-1}\ s^{-1}$)
DNA	8×10^8
Linoleic acid	9×10^9
Collagen	4×10^{11}
Albumin	8×10^{10}
Cysteine	3.4×10^{10}
Methionine	8.3×10^9
Tryptophan	1.3×10^{10}
Histidine	1.3×10^{10}
Tyrosine	1.3×10^{10}
Arginine	3.5×10^9
Ascorbate	1×10^{10}
Trolox	6.9×10^9

Adapted from Davies, M.J. 2005. The oxidative environment and protein damage. Biochim. Biophys. Acta. 1703, 93–109.

"sacrificial loss," by which amino acids of negligible biological significance would scavenge ROS and as a result, defend valuable residues and biomolecules (including unsaturated lipids) against oxidation (Davies, 2005). When this antioxidant protection is consumed, lipids and proteins may undergo oxidative deterioration.

LOX consists of a free radical chain reaction that leads to the degradation of an unsaturated fatty acid in three steps: initiation, propagation, and termination (Min and Ahn, 2005). ROS initiate the chain reaction by abstracting a hydrogen atom from an unsaturated fatty acid (RH) to form a free radical lipid (alkyl radical, $R^{\bullet}$). Diverse radical species such as peroxyl radicals (ROO^{*}) are subsequently formed upon reaction of $R^{\bullet}$ with molecular oxygen. These radicals propagate the reaction to neighboring RH yielding a lipid hydroperoxide (ROOH) and a new $R^{\bullet}$. Eventually, radical species find stability in succeeding degradation steps that lead to the formation of nonradical molecular weight compounds. Volatile compounds, including alcohols, acids and carbonyls are among the most abundant LOX products in poultry and processed-poultry foods. Aldehydes such as hexanal, malondialdehyde (MDA) and 4-hydroxy-2-trans-nonenal (4-HNE) are commonly used as reliable LOX indicators (Ladikos and Lougovois, 1990).

As for LOX, PROTOX is typically mediated by ROS and manifested as a radical-mediated chain reaction (Davies, 2005). While the underlying mechanisms and the oxidation products are fairly different between LOX and PROTOX, lipids and proteins interact during the oxidative reactions so that reactive species may be transferred between biomolecules (Lund et al., 2011). Like this, radicals and hydroperoxides formed from RH may attack susceptible amino acid side chains and trigger PROTOX. Final lipid oxidation products such as the aforementioned MDA and 4-HNE have been found to form complexes with food proteins via carbonyl-amine reactions (Zhou et al., 2014, 2015). The formation of these complexes affects the physicochemical properties, functionality and digestibility of meat proteins (Zhou et al., 2014, 2015).

When mediated by ROS, the chemistry behind PROTOX is greatly dependent on the target in the protein (Davies, 2005). The attack of ROS on the peptide backbone leads to peptide scission while the oxidation of the amino acid side chains leads to the formation of specific degradation products. For instance, methionine is oxidized into methionine sulfoxide, tryptophan oxidation leads to the formation of kynurenine derivatives and alkaline amino acids (lysine, arginine, threonine, and proline) are deaminated to yield carbonyls (Davies, 2005). The oxidation of cysteine and tyrosine leads to the formation of protein cross-links (disulfide bonds and dityrosines, respectively) (Davies, 2005). In meat systems, the loss of tryptophan and thiols are regarded as early events of the oxidative damage to proteins while the formation of cross-links and protein carbonyls occur in more advanced stages (Lund et al., 2011; Soladoye et al., 2015). As for LOX products, some PROTOX oxidation products such as protein carbonyls are highly reactive

and may be implicated in further reactions. The α-aminoadipic semialdehyde, a major protein carbonyl, may also be involved in advanced reactions including Strecker-type degradation of amino acids and carbonylamine additions upon reaction with other alkaline amino acids (Estévez, 2011). For an in-depth revision of lipid and PROTOX mechanisms and pathways, comprehensive reviews are available (Ladikos and Lougovois, 1990; Estévez, 2011; Lund et al., 2011; Bekhit et al., 2013; Soladoye et al., 2015)

6.3 COLOR OF POULTRY: BIOCHEMICAL BASIS

Transformation of muscle into meat involves a series of chemical and biochemical steps that affect the color displayed by poultry meat (Petracci and Fletcher, 2002). In particular, poultry-meat color is dependent on the concentration and chemical state of meat pigments, especially: Mb, hemoglobin, cytochrome C and their derivatives, presence of ligands complexing with heme pigments (Fletcher, 2002). The physical characteristics of the meat, such as dispersion and absorption of light, also influence color disposition (Kropf, 1993). With regard to consumer acceptability of poultry meat color, the tan color of cooked poultry meat is of particular relevance, and is dependent on assorted biochemical and processing factors such as, once again, the concentration and chemical state of the Mb, nitrosative reactions, irradiation, cooking temperature, storage, canning, processing additives, preslaughter conditions, and hemachromes, and cytochrome C reactions (Maga, 1994).

Mb is the main protein responsible for the meat color and has a fundamental contribution in the color of beef, pork, lamb, and poultry (Mancini and Hunt, 2005). The biochemical structures that define the color of Mb in mammals and birds have very similar composition (Table 6.2), but the

TABLE 6.2 Percentage Amino Acid Sequence Similarities Between Myoglobins From Poultry Meat and Red Meat Sources

Species	Chicken	Turkey	Ostrich	Emu	Beef	Sheep	Pig
Chicken	100						
Turkey	100	100					
Ostrich	92.8	92.8	100				
Emu	90.2	90.2	94.8	100			
Beef	72.5	72.5	70.6	69.3	100		
Sheep	72.5	72.5	70.6	69.3	98.7	100	
Pig	76.5	76.5	74.5	73.2	88.2	89.5	100

Adapted from Suman, S.P., Joseph, P., 2013. Myoglobin chemistry and meat color. Annu. Rev. Food Sci. Technol. 4, 79–99.

chemical structures and Mb functions differ between muscle (in vivo) and meat (postmortem) as discussed by Suman and Joseph (2013). The authors found that the amino acid sequence of chicken Mb is the same as that for turkeys. Mb is formed by a polypeptide chain, the globin, with numerous histidine residues that have received considerable attention due of its key role in the structure and Mb function (Mancini and Hunt, 2005). Mb also contains a prosthetic group that is located inside its hydrophobic portion, the heme group. The globin chain confers water solubility to heme and protects the heme iron from oxidation, therefore the protein can maintain its functionality (Suman and Joseph, 2013). The heme iron ring features an atom centrally located that can form six coordinate bonds. Four of the bonds are pyrrole groups of the heme porphyrin ring, while the fifth bonds to proximal histidine-93. The sixth link is available for binding to the various forms of reversible binders such as oxygen and nitric oxide (NO) (Cornforth and Jayasingh, 2004). In in vivo muscle, the Mb exerts a function as binder to oxygen and also provides oxygen to the mitochondria, allowing the tissue to maintain its physiological functions, i.e., cellular homeostasis (Wittenberg and Wittenberg, 2003). On the other hand, in meat, Mb is the major pigment responsible for the red color. The ability to bind oxygen is due to the presence of the heme group located inside the chain (Suman and Joseph, 2013). The illuminant nature of conjugated double bonds of the heme group is responsible for the capacity of absorbing visible light and thus its function is to serve as a pigment.

The fresh meat color is defined by the relative amount of four redox states of Mb, namely deoxymyoglobin (DeoxyMb), oxymyoglobin (OxyMb), carboxymyoglobin (COMb), and metmyoglobin (MetMb) (Fig. 6.1). OxyMb and COMb provide the cherry-red and the red colors, respectively, of these two redox forms and they are indistinguishable by the human eye (Cornforth

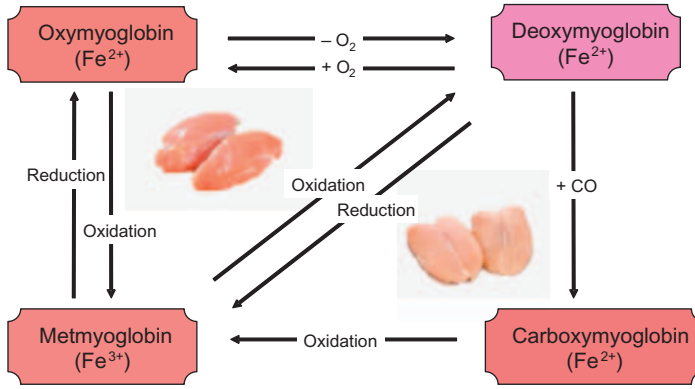


FIGURE 6.1 Myoglobin redox forms in poultry meats. *Adapted from Mancini, R.A., Hunt, M.C., 2005. Current research in meat color. Meat Sci. 71, 100–121.*

and Hunt, 2008). DeoxyMb is purplish-red in color. The sixth coordinate heme iron is occupied by oxygen in OxyMb and CO in COMb (Von Seggern et al., 2005), whereas no linking is bound with the heme iron in DeoxyMb. Saturating Mb with oxygen (O_2) provides cherry-red (OxyMb). Formation of MetMb (brown) results from the oxidation of the three ferrous (Fe^{2+}) forms to a ferric (Fe^{3+}) state and is associated with poultry meat discoloration. MetMb has a water molecule bound at the sixth coordinate of the ferric heme and is incapable of binding oxygen (Suman and Joseph, 2013).

The relationship between animal species, muscle type, and animal age on meat Mb content and visual color was reported by Miller (1994). With regard to poultry meat in particular, white meat from 8-week-old poultry had the lowest Mb content (0.01 mg Mb/g meat) followed by 26-week-old male poultry white meat (0.10 mg/g), young turkey white meat (0.12 mg/g), 8-week-old poultry dark meat (0.40 mg/g), 26-week-old male poultry dark meat (1.50 mg/g), and 24-week-old male turkey dark meat (1.50 mg/g). These values are considerably lower than Mb concentrations reported in other animals species such as young lamb (2.50 mg/g), dark meat fish species (5.3−24.4 mg/g), 3-year-old beef (4.60 mg/g), and old beef (16−20 mg/g). Knowing and controlling the genetic, processing, and environmental factors having an impact on poultry color allow manipulation of color in raw as well as in processed poultry meat product in order to satisfy consumer's expectation. The biochemistry principles behind the endogenous and exogenous factors have been exploited to develop strategies in meat processing and animal production to minimize discoloration and possible defects and enhance desirable color attributes (Fletcher, 2002; Bianchi et al., 2007; Estévez, 2015). Among the latter, Bianchi et al. (2007) reported a positive correlation (0.92) between the yellowness of the skin and breast meat, with both conditions being highly appreciated by poultry consumers (Fletcher, 2002). According to Fletcher (2002), deposition of yellow pigments (carotenoid) in the skin of the bird is influenced by factors such as the genetic capability of the bird, dietary source of pigments, health of the bird, and processing.

6.4 OXIDATIVE DAMAGE AND DISCOLORATION OF POULTRY

6.4.1 Oxidation of Poultry

Poultry and poultry meat are particularly affected by oxidative reactions and the formation in the food chain may have consequences for the consumer health (Estévez, 2015). Various oxidation-processing steps affect the physiology and physicochemical properties of poultry, compromising the animal growth, performance, and ultimately the food quality. Protein and lipid oxidation has been recognized as a major threat to the quality of processed

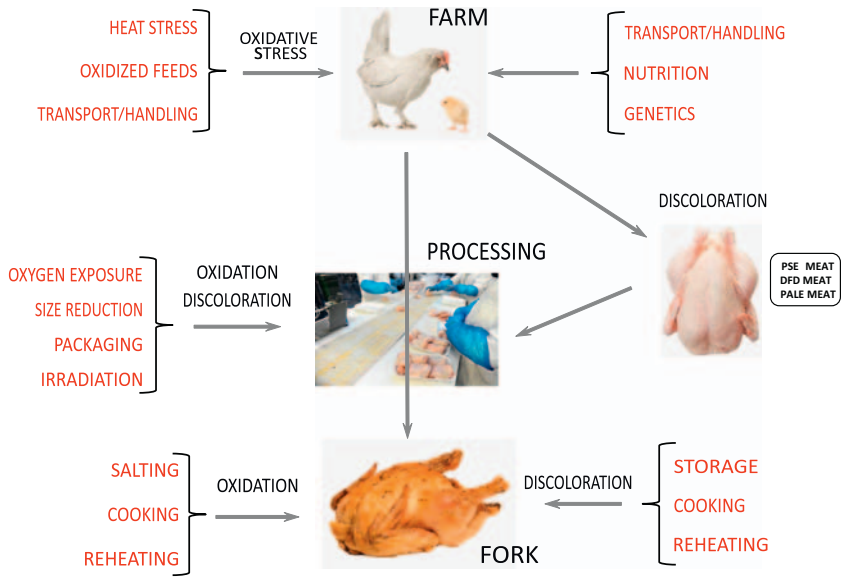


FIGURE 6.2 Sources of oxidative stress and discoloration to poultry and poultry meat from farm to fork.

poultry products. Currently, there is a growing interest among scientists and producers to explore the effective ways to reduce the oxidative response and to improve poultry meat quality. Additionally, the oxidative damage also is an important mechanism of biological injury in domestic birds and a leading cause of pathologies (Fellenberg and Speisky, 2006).

Estévez (2015) reported that oxidation in birds is induced by oxidative stress from the farm to the fork (Fig. 6.2). This chapter explores the relevance and consequences of the oxidative reactions throughout the production of poultry meat. The development of oxidation in each of the food chain steps from farm to food consumption has been well illustrated by Estévez (2015). The susceptibility of poultry oxidation is believed to have diverse factors origin, in particular due to the birds' genetic selection toward larger breast muscles production, consequently increasing total weight, and faster growth rates (Mujahid et al., 2005; Sandercock et al., 2006; Kuttappan et al., 2016). This intense genetic selection makes domestic birds like broilers particularly susceptible to oxidation (Kuttappan et al., 2016). Works from Zhang et al. (2011) and Ismail et al. (2013) reported that poultry production in hot climates under oxidized feeding worsen the in vivo oxidative stress in chicken tissues. Others authors reported that feeding broilers with oxidized vegetable oil led to an increase levels of malonaldehyde in blood plasma and in breast meat compared to birds fed fresh oil (Engberg et al., 1996; Delles et al., 2014). Zhang et al. (2011) investigated the effects of supplementing

oxidized oil or antioxidants in diet on the oxidative stress of animals and on the quality of fresh broiler breast meat. The authors suggested that dietary oxidized oil induced oxidative stress in live broilers and increased lipid and protein oxidation in breast muscle. Furthermore, Delles et al. (2014) evaluated the influence of dietary antioxidants and quality of oil on the oxidative and enzymes properties of chicken broiler breast meat and showed that feeding diets with high oxidized oil besides increasing the vulnerability of lipids and proteins to oxidation can reduced the activities of tissue antioxidant defense enzymes: catalase (CAT), glutathione-peroxidase (GSH-Px), and sodium dismutase (SOD). Oxidative reactions increased as a result of a high consumption of oxidized lipids (Zhang et al., 2011; Delles et al., 2014) and either oxidation of sensitive polyunsaturated fatty acids (PUFAs) or prooxidants (Bekhit et al., 2013). Wang et al. (2009) and Ismail et al. (2013) reported that poor handling due the exposition of preslaughter stress on broilers by heat exposure led muscles to high susceptibility to lipid and protein oxidation, and diverse pathologies. Unfortunately, providing better handling, such as cooling the birds in animal houses, in most cases is financially expensive and difficult to implement. Nevertheless, some activities have been carried out in order to provide a better conditions for the animals. There is a favorable consequence to the carcass weight gain by growing the animals in a dark house, and this seems to be a potential maneuver to be explored commercially on farms (Carvalho et al., 2015). Also, providing a water shower to the birds immediately before transport which is associated with an improvement in air velocity throughout the truck container (Spurio et al., 2015), and water nebulization and holding time period at the slaughterhouse (Guarnieri et al. 2004) are antemortem maneuvers that improve animal welfare, and consequently meat quality.

Subsequently to the production step, Wang et al. (2015a) evaluated the effect of dietary supplementation with creatine monohydrate (CMH) during the finishing period on the muscle lipid peroxidation and antioxidant capacity of broilers that experienced transport stress in the summer season. The results showed that transport stress accelerated muscle lipid peroxidation by increasing TBARS production and increases antioxidants compounds (SOD, GSH-Px, peroxisome proliferator-activated receptor γ coactivator-1α (avPGC-1α), and heat shock protein 70 (HSP70) were insufficient to reduce muscle TBARS and prevent muscle from transport-induced oxidative stress.

Also as reported by Cheah et al. (1995) in pigs and by Olivo et al. (2001) in broilers the dietary supplementation of vitamin E is capable of inhibiting the development of the physical stress by impairing the phospholipase A_2 (PLA_2) activity and therefore long-chain unsaturated fatty acids are not liberated giving rise to the prolonged stability of mitochondrial membranes, hindering leakage of Ca^{2+} ions. Release of Ca^{2+} from the sarcoplasmic reticulum induced rapid glycolysis and under these conditions the meat protein functional properties were harmed (Carvalho et al., 2014; Pedrão et al., 2015).

Upon slaughter, the development of early postmortem lipid and protein oxidation in birds has been reported during chilled storage of chicken (Zhang et al., 2011), turkey (Chan et al., 2011), rhea (Filgueras et al., 2010), and ostrich meat (Leygonie et al., 2011), and also during the processing of chicken (Ali et al., 2015) and turkey (Wang et al., 2012). The birds are more susceptible to the oxidation process than pork and beef during frozen storage (reviewed by Leygonie et al., 2012; Utrera and Estévez, 2013). The high levels of unsaturation of poultry meat lipids is also responsible for the susceptibility of meat oxidation, but other compounds such as endogenous enzymes and heme and nonheme iron content is also influential (Min et al., 2008; Utrera and Estévez, 2013; Estévez, 2015).

The postrigor lactate production facilitates the oxidation of muscle components as hydrogen (H^+) can lead to pro-oxidant action by the redox cycle of Mb (Estévez, 2011, 2015). Another component that influences biochemical changes is the free-catalytic iron which favors the oxidizing enzymes and also contributes to promoting lipid and protein oxidation in postrigor meat. The oxidative damage to meat proteins during frozen storage seems to be linked to poor meat quality due to biochemical reactions which are influenced by the muscle types and freezing temperature (Leygonie et al., 2012). On the other hand, heme pigments, especially Mb, catalyze the lipid oxidation in meat. Other heme compounds also contribute as pro-oxidants in muscle tissue (Love, 1983; Han et al., 1994). Baron et al. (1997) reported that MetMb acts as a pro-oxidant at acidic pH and in the presence of hydroperoxides.

As a consequence of low meat pH, the denaturation of protein leads to an increased susceptibility of Mb to autoxidation and to the subsequent change of color during refrigerated storage post freeze/thaw (Abdallah et al., 1999; Leygonie et al., 2011; Leygonie et al., 2012). Lipid and protein oxidation results from the formation of pro-oxidant substances that are reactive with meat pigments leading to OxyMb to MetMb development (Farouk and Swan, 1998). When the oxidation reactions are accelerated by frozen storage or during meat processing, the quantity of free radicals present is increased, leading to an increased rate of Mb oxidation and a decrease in color stability (Wang et al., 2009; Ali et al., 2015).

6.4.2 Impact of Oxidation on Poultry Quality

Oxidation has indisputable negative consequences on the quality of poultry meat whether the oxidative damage occurs in vivo or in the meat after slaughter. In fact, the oxidative stress in living tissues not only affects the production rates and health status of birds, in vivo LOX and PROTOX also have consequences for meat quality (Estévez, 2015). While some of these quality defects are the subject of current and multidisciplinary studies, scientific studies suggest the implication of in vivo oxidative stress on the incidence of white striping (Kuttappan et al., 2013), pink color defect (Holownia

et al., 2003), and wooden breast (Sihvo et al., 2014) in domestic birds. Oxidative stress may also increase meat toughness by decreasing collagen turnover by intramuscular fibroblasts leading to decreased collagen solubility (Archile-Contreras and Purslow, 2012). The oxidation of proteins in vivo has been found to impair the water-holding capacity (WHC) of chicken meat (Zhang et al., 2011). Chan et al. (2011) observed a clear association between ultimate pH, coloring of turkey breast, and susceptibility of muscle proteins to oxidation, including consequences on WHC. The effect of antemortem physiopathological conditions on oxidative stress and the impact of that on poultry quality require further elucidation.

Intense and persistent LOX during handling, processing and storage of poultry meat leads to severe quality deterioration including impairment of flavor, color, and texture (Min and Ahn, 2005). A major concern in processed poultry products is the onset of rancidity and off-odors as a result of LOX occurring in cooked and reheated products (Ladikos and Lougovois, 1990; Min and Ahn, 2005). In particular, cooked meats are known to develop a fast and intense flavor deterioration during the subsequent cold storage commonly described as warmed-over-flavor (WOF) (Pegg and Shahidi, 2012). This unpleasant flavor, which may be responsible for the consumer rejection and relevant economic losses, is linked to the formation of specific lipid-derived odor-active volatiles such as pentanal, hexanal, and alkadienals (Jayasena et al., 2013). This deterioration process is particularly relevant in poultry products owing to their high degree of fat unsaturation and high susceptibility to oxidative reactions (Pegg and Shahidi, 2012). Other factors such as endogenous enzymes and heme and nonheme iron contents may also be influential (Min et al., 2008; Utrera and Estévez, 2013). In agreement with previous reports, Utrera et al. (2014) found that when exposed to an intense prooxidative environment, such as that created during heat treatment, chicken meat shows a higher susceptibility to LOX than pork and beef. Furthermore, the intense LOX was found to greatly affect proteins, manifested as intense carbonylation and formation of protein cross-links. These chemical changes were thought to be responsible for a dramatic deterioration of several quality traits including loss of WHC and increased hardness. The connection between PROTOX in aging, chilling, and frozen storage of postmortem muscle and impaired protein functionality (including WHC) has been proposed in numerous studies (Zhang et al., 2011; Leygonie et al., 2012; Utrera and Estévez, 2012). A higher water loss upon thawing of frozen meat and its subsequent cooking may, in turn, affect other quality traits such as color and texture properties of meat (Utrera et al., 2014). PROTOX has also been reported to be responsible for increased meat toughness via two likely mechanisms. The first may involve the inactivation of the calpain system which causes an impaired tenderization of the aging muscle (Huff-Lonergan et al., 2010). The second consists of a physical strengthening of the muscle structure through the formation of oxidative protein cross-links (Lund et al., 2011).

6.4.3 Color Defects and Discoloration of Poultry

The color of poultry meat can vary from a bluish-white to yellow when raw. All of these colors can be normal and are a direct result of species, type of muscle, exercise, age, and/or diet. Younger birds have low fat under the skin, which can lead to a bluish tint, and the yellow skin and meat could be a result of pigments in the feed (Brown, 2014). Discoloration is associated with oxidative damage to poultry and can cause meat defects during poultry production and handling on the farm as well as during meat processing: chilling, storage, cooking, and others (Fig. 6.2). The impairment of the homeostasis of lipids and proteins leads to oxidation in muscle promoting changes in meat color due to modification of pigments state, as mentioned above (Zhang et al., 2011; Xiao et al., 2011a; Estévez, 2015).

Currently, there is a large selection of birds for growth, feed conversion, and white meat yield (Havenstein et al., 2003). Unfortunately, this progressive diversification has resulted in increased atypical poultry meat (Harford et al., 2014). This atypical meat includes pale, soft, and exudative (PSE)-like and dark, firm, and dry (DFD)-like meats with these abnormalities changing the expected color of meat mostly because of poor preslaughter handling (Fletcher, 2002; Petracci et al., 2009; Dadgar et al., 2012; Estévez, 2015). Consolidated knowledge as well as some recent studies include oxidative reactions as a driving biochemical mechanism behind the quality impairment of PSE and PSE-like poultry meat. Through the catalytic action of metal ions, in the presence of various factors such as light, increased temperature, and oxygen, fatty acids increase the unstable conditions toward autoxidation reactions (Bekhit et al., 2013). Soares et al. (2003) postulated that thermal stress promoted higher mitochondrial PLA_2 activity in the presence of higher concentrations of Ca^{2+} and that this could be the triggering factor in initiating the characteristic symptoms, leading to broiler PSE meat formation (Fig. 6.3). The results of a recent experiment have indicated that protein oxidation plays a predominant role during the biochemical reactions within the PSE meat installation as PSE breasts samples are more susceptible to

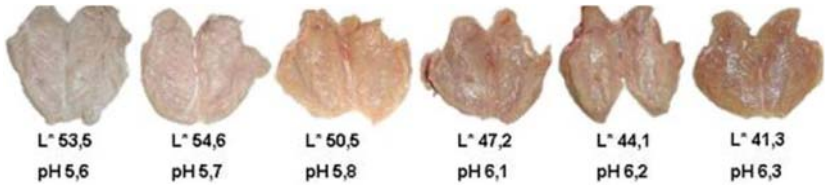


FIGURE 6.3 Broiler breast discoloration related to L* values and final pH. *Adapted from Oda, S.H.I., Nepomuceno, A.L., Ledur, M.C., Oliveira, M.C.N., Marin, S.R.R., Ida, E.I., Shimokomaki, M., 2009. Quantitative differential expression of alpha and beta ryanodine receptor genes in PSE (pale, soft, exudative) meat from two chicken lines: broiler and layer. Braz. Arch. Biol. Techn. 52, 1519–1525.*

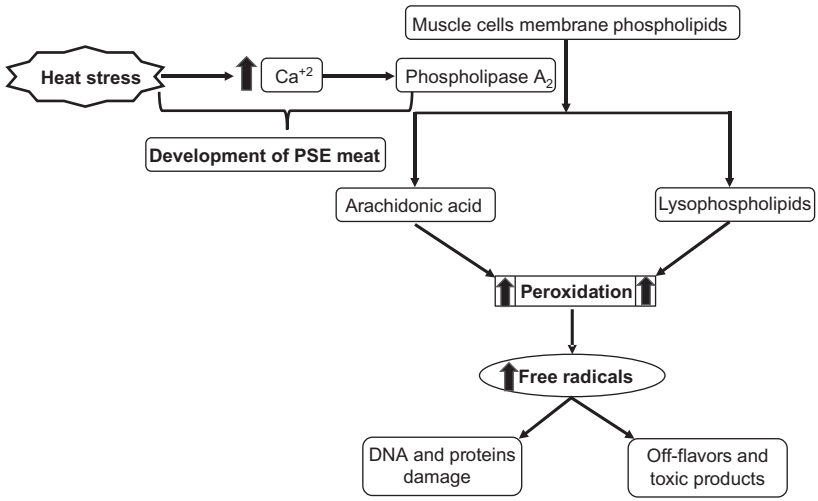


FIGURE 6.4 Development of pale, soft, and exudative meat associated with arachidonic acid metabolism through phospholipase A_2 action over muscle cell membranes. *Adapted from Soares, A.L., Marchi, D.F., Matsushita, M., Guarnieri, P.D., Droval, A.A., Ida, E.I., Shimokomaki, M., 2009. Lipid oxidation and fatty acid profile related to broiler breast meat color abnormalities. Braz. Arch. Biol. Technol. 52, 1513–1518.*

oxidative stress due to lower concentrations of antioxidant enzymes (CAT, GSH-Px, and SOD) parallel to the pro-oxidant activity of PLA_2 enzyme. PSE was susceptible to protein oxidation during chilled storage with more intense tryptophan and thiols depletion, higher protein carbonylation, and more intense formation of protein cross-links (Fig. 6.4). The consequence is the oxidative damage to meat proteins contributing to the loss of protein functionality impairing the WHC of PSE meat (Carvalho et al., 2017).

Others alterations in meat discoloration are influenced by animal nutrition (Wang et al., 2015a; Zhang et al., 2011), handling (Carvalho et al., 2015), and carcass cooling (Chan et al., 2011) and storage (Filgueras et al., 2010, 2011; Xiao et al., 2011a; Lázaro et al., 2014) in assorted birds species such as broilers (Wang et al., 2009), turkey (Chan et al., 2011), rhea (Filgueras et al., 2010, 2011), and laying hens (Jin et al., 2015). Xiao et al. (2011a) evaluated in thighs the effects of the addition of antioxidants or oxidized oil to the diets, use of irradiation, and different types of packaging on the oxidative stability. Results showed that dietary treatment with vitamin E and oxidized oil can directly influence the lipid and protein oxidation of chicken meat during refrigerated storage. The use of supplementation (vitamin E) improved the color L^* (lightness) value, but irradiation improved the a^* (redness) value of chicken thigh meat. However, Zhang et al. (2011), despite higher levels of occurrence of protein and lipid oxidation, found no difference in color L^* (lightness), a^* (yellowness), and b^* (brownness) values of

breast muscle from three diets: 1—control; 2—oxidized diet (5% oxidized oil); and 3—500 IU vitamin E. Rababah et al. (2010) compared the effect of green tea extract and commercial grapefruit seed extract extracts, and tertiary butylhydroxyquinone (TBHQ) on the physicochemical properties of irradiated fresh boneless chicken breast meat during storage. The effect of irradiation on the lightness value (L^*), decreased depending on storage time and infusion treatment. The use of antioxidants and plant extracts was effective in preventing physicochemical changes in chicken breast meat caused by irradiation. Finally, Ali et al. (2015) studied the impact of freeze−thaw cycles on protein stability and its relationship with lipid and protein oxidation and changes of color in chicken meat muscles. The authors reported that increasing the number of freeze−thaw cycles might increase the lipid and protein oxidation, together with protein denaturation and lead to the loss of muscle water and a decrease in the color stability of chicken meat.

6.5 ANTIOXIDANT PROTECTION OF POULTRY: FUTURE PERSPECTIVES

6.5.1 Antemortem Strategies

Given the high susceptibility of poultry to oxidative damage and the overall negative influence of uncontrolled oxidative reactions on poultry quality, antioxidant strategies may be designed to protect both the living animal and the obtained products (Table 6.3). In this regard, antemortem strategies are usually aimed at improving the oxidative stability of poultry through the manipulation of diet and feeds. Dietary antioxidants may even ease the oxidative stress caused in broilers by environmental factors (i.e., heat exposure) (Ismail et al., 2013). The supplementation with α-tocopherol (around 200 mg tocopherol/kg feed) and ascorbate (up to 1000 mg ascorbate/kg feed) have been found to be effective against LOX, and PROTOX (Hu et al., 2015a). The beneficial effect of supplemented vitamin E to inhibit lipid oxidation, to stabilize meat color (Buckley et al., 1995), and to decrease the drip loss (Jensen et al., 1997) is well established. This fact was attributed to the stabilizing effect of α-tocopherol on membrane fluidity and integrity (Monahan et al., 1994). While these dietary supplements are generally beneficial against discoloration, some inconsistent results are found in the literature as some authors found antioxidant protection on lipids and proteins and no apparent effect on color (Zhang et al., 2011). Therefore, a larger protection may imply the combination of the aforementioned additives with other micronutrients such as selenium, magnesium, and zinc (Surai, 2002; Young et al., 2003; Guo et al., 2003; Xiao et al., 2011b; Skrivan et al., 2012; Ismail et al., 2013; Panda and Cherian, 2014; Naz et al., 2016). Selenium as well as methionine supplementation has been largely recognized to inhibit LOX, and to lead to a loss of WHC and discoloration in poultry muscle (Wang et al.,

TABLE 6.3 Strategies Aimed to Reduce Oxidative Stress and Discoloration in Assorted Poultry Products

Strategy	Poultry System	Overall Effect	Reference
Antemortem			
α-Tocopherol supplementation	Chicken broilers	Antioxidant defense, better performance	Hu et al. (2015a)
Met and Se supplementation	Maternal line	Improved oxidative and color stability of progeny breasts	Wang et al. (2009)
Dietary resveratrol	Assorted poultry	Antioxidant protection	Alagawany et al. (2015)
Apple peel	Chicken broilers	Antioxidant protection	Heidarisafar et al. (2016)
Packaging			
Rosemary active packaging	Breast chicken	Antioxidant protection	Bolumar et al. (2011)
High-oxygen packaging	Chicken thigh	Pro-oxidant	Jongberg et al. (2014)
Vacuum packaging	Chicken thigh	Antioxidant protection	Xiao et al. (2011a)
Product Formulation			
Green cabbage	Chicken meatballs	Antioxidant protection	Verma et al. (2016)
Sapodilla/ banana extracts	Chicken patties	Antioxidant protection	Devatkal et al. (2014)
Thyme/orange	Chicken muscles	Antioxidant protection	Rimini et al. (2014)
Rosemary	Frozen chicken nuggets	Antioxidant protection	Rocío Teruel et al. (2015)
Citrus extract	Cooked turkey meat	Antioxidant protection	Contini et al. (2014)
Lactic acid bacteria isolated	Experimental sausage	Antioxidant protection	Chen et al. (2015)

2009). Other amino acids such as histidine have also been proposed to be included in poultry's diet to improve their antioxidant status (Kopec et al., 2016). Carnitine (Surai, 2015), creatine (Wang et al., 2015a), α-lipoic acid (Khan et al., 2015), conjugated linoleic acid (Jiang et al., 2014), chitosan (Li

et al., 2016), and assorted phytogenics (Surai, 2015), including pure phenolic compounds such as resveratrol (Alagawany et al., 2015), have been recently proposed to be included in animal feeds with antioxidant purposes. Regarding the latter, dietary strategies based on phenolic-rich plant materials have been found to be effective against LOX and PROTOX in various poultry meat and processed poultry products including tea catechins (DeSmet et al., 2008), apple peel (Heidarisafar et al., 2016), grape pomace (DeSmet et al., 2008), tomato extract (DeSmet et al., 2008; Vossen et al., 2011), pea seeds (Laudadio and Tufarelli, 2011), rosehips and rosemary leaves (Loetscher et al., 2013), and assorted vegetables' powder (Duthie et al., 2013). In addition to their antioxidant properties, certain dietary phytogenics could contribute to other physiological benefits including digestibility of growing broilers (Cross et al., 2007). Supported by favorable results, the application of dietary phytogenics has been recently suggested as an alternative to antibiotics to control oxidative stress in broiler chicks (Sethiya, 2016). Some studies, however, report that dietary natural sources of phytochemicals may not be as effective as synthetic antioxidants for protecting meat from broilers (DeSmet et al., 2008).

6.5.2 Packaging Strategies

Considerable advances have also been gained in the last years in relation to meat packaging with the purpose of minimizing oxidative reactions and, hence, controlling their potential negative effects on poultry quality. Antioxidant strategies based on packaging typically involve oxygen exclusion and skin vacuum packaging have been reported as efficient in protecting poultry meat against LOX and PROTOX (Clariana et al., 2012; Xiao et al., 2011a,b). According to Bolumar et al. (2011), the incorporation of 10% rosemary extract to an active packaging system delayed the oxidative reactions induced in breast chicken by high-hydrostatic pressure. While high-oxygen packaging seems to be required to keep a desired fresh red meat color in beef and some pork cuts, for white meats this may be unnecessary given the low Mb concentration. On the other hand, the employment of vacuum packaging is suitable for inhibiting protein and lipid oxidation in broiler (Xiao et al., 2011a), rhea (Filgueras et al., 2010), and turkey (Fraqueza and Barreto, 2011) meat. According to Fraqueza and Barreto (2011) only modified atmosphere packaging (MAP: 100% N_2), without CO_2 or CO, prevented lipid oxidation in turkey meat. Additionally, the presence of CO in anoxic gas mixtures with CO_2 for turkey meat improved the bright-pink color preferred by consumers. However, in practice, some industries keep on using high-oxygen modified atmosphere in meat and some viscera from broilers leading to unsatisfactory results, including oxidation and color problems. A recent study reported that the susceptibility of chicken meat to high-oxygen packaging depends on the muscle type, with thigh being more

susceptible than breast (Jongberg et al., 2014). The increase of carbonyls, the loss of thiols and the formation of protein cross-links may be responsible for the increase of toughness reported by Jongberg et al. (2014) in chicken meat packed in high oxygen in accordance with the mechanisms reported in the previous section. For goose or duck meat, high oxygen may be recommended, and some authors have reported that packing in a high-oxygen atmosphere (consisting of 80% O_2, 20% CO_2) maintained the red color of goose breast meat for 11 days of chilled storage (Orkusz et al., 2013). For other birds such as rhea meat, the exposure to air was reported to be particularly harmful to the quality of red muscles while vacuum packaging protected against Mb, lipid, and PROTOX (Filgueras et al., 2010).

6.5.3 Formulation Strategies

As an alternative to the usage of conventional antioxidant additives in meat products, the search for alternative sources of antioxidant compounds in the plant kingdom is currently a topic of growing interest (Falowo et al., 2014). Plant phenolics have been shown to be effective at reducing lipid oxidation and rancidity and extending shelf life in a large variety of poultry-based foods (Bekhit et al., 2013; Falowo et al., 2014). Today, the number of natural sources of bioactive compounds tested in meat products is enormous and includes extracts from grape seed (Gómez et al., 2014), citric fruits (Contini et al., 2014), tea (Ye et al., 2015), sapodilla and banana extracts (Devatkal et al., 2014), rosemary (Rocío Teruel et al., 2015), thyme (Rimini et al., 2014), and green cabbage (Verma et al., 2016), just to mention a few. In some cases, the effectiveness of these natural antioxidants is compared with that of common additives such as ascorbate and/or synthetic antioxidants, and is used to counteract the pro-oxidant effect of particular technologies such as irradiation or high-hydrostatic pressure (Rababah et al., 2010; Bolumar et al., 2011; Jin et al., 2015). In addition to the natural source, their content of bioactive compounds and the dose and application mode have become issues with remarkable influence on the antioxidant outcome. While the direct addition as powder or extract to comminuted meat products is simple and popular (Armenteros et al., 2013; López-Vargas et al., 2014), marination, spraying, and injection/enhancement have been tested for whole meat products (Rimini et al., 2014; Armenteros et al., 2016). Innovatively, bioactive compounds and antioxidant additives have also been incorporated in microcapsules (Sickler et al., 2013), edible films (Ruiz-Navajas et al., 2015), polyethylene terephthalate (PET) trays (Contini et al., 2014), and chitosan nanoparticles (Hu et al., 2015b). The so called "natural antioxidants" may not only be found in plant materials, as diverse meat products and their hydrosylates have been recognized as sources of peptides with antioxidant potential (Wang et al., 2015b). Other innovative means to protect against oxidative reactions in meat products include Maillard advanced reaction

products (Miranda et al., 2012) and inoculation with lactic acid bacteria (Chen et al., 2015). However, certain compounds that are able to prevent lipid oxidation may not be effective at inhibiting PROTOX (Estévez, 2011). The overall effect of plant phenolics against PROTOX is governed by specific interaction mechanisms between the phenolic compounds and food proteins that may lead to anti- or pro-oxidant actions. Further information about these mechanisms, which may not be applied to meat lipids, can be found elsewhere (Lund et al., 2011; Estévez, 2011). Another example of a technological strategy with contradictory effects on lipids and proteins is NO. According to studies (Wang et al., 2009) chicken breasts in which NO was enhanced had higher LOX levels and discoloration but lower protein carbonylation than control samples. Conversely, Zhang et al. (2013) reported opposite results including higher PROTOX and impaired WHC in chicken breasts in which NO was increased. As already mentioned, the role of RNS in regulating oxidative reactions in poultry and poultry products should be further investigated.

6.6 CONCLUSION

As the genetic pressure on domestic birds increases for faster growth and larger breast muscles, the occurrence of quality defects in which oxidative stress plays an underlying role also appears to be a growing problem. The classical threat of oxidation to poultry quality in terms of discoloration, rancidity, and product rejection joins with novel quality shortcomings such as white striping, wooden breast, and other unusual poultry defects. The necessity to minimize the occurrence of these problems in a society with increasing awareness of food as a source of well-being and lifespan, urges the scientific community to develop innovative means to alleviate oxidative stress in living birds and poultry products. Thus, it seems unavoidable that studies that probe deeper into the fundamentals of animal nutrition, genetics, well-being, and the biochemical basis of all these new quality defects are carried out.

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Chapter 7

Current Challenges in Poultry Meat Safety

Frederique Pasquali[1], Alessandra De Cesare[1], Marine Meunier[2], Muriel Guyard[2], Katell Rivoal[2], Marianne Chemaly[2] and Gerardo Manfreda[1]

[1]*University of Bologna, Bologna, Italy,* [2]*French Agency for Food, Environmental and Occupational Health Safety (Anses), Ploufragan, France*

7.1 INTRODUCTION

Poultry and poultry meat might harbor food-borne bacterial pathogens. European and International epidemiological reports confirm *Salmonella* and *Campylobacter* as major risks of food-borne zoonosis. Recently, due to improvements in isolation and identification methods, Arcobacter and *Helicobacter pullorum* were described as emerging pathogens previously under-reported. The gut microbiota is one of the main defence components in the gastrointestinal tract (GIT) against enteric pathogens. Disturbance of the gut microbiota−host interaction plays a crucial role in the development of intestinal disorders. Until recently, those interactions were mostly unknown due to the predominance of uncultivable species of the gut microbiota. Recent advances in nonculture-based approaches such as metagenomics boost those studies by collectively investigating sets of genomes from the microbiota. Along with biological hazards, a current challenge worldwide is the antibiotic treatment failure against bacterial infections. This failure has been linked to the overuse of antimicrobial drugs in animal production and humans, suggesting poultry production as one of the reservoirs of antimicrobial resistance (AMR) genes and bacteria. In recent years, different strategies to face these risks have been studied and implemented, both on live poultry and on poultry meat. Strategies at farm level, such as biosecurity, vaccination, feed additives, as well as at the slaughterhouse, such as poultry carcass decontamination (chemical and physical treatment), are reviewed along with new genomics approaches focused on improving the gut health and the disease resistance of animals. Moreover, new risk-based metrics such as the Appropriate Level of Protection (ALOP), Food Safety Objective (FSO), and Performance Objective (PO), are described as new

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concepts to increase the food safety for consumers. Therefore a holistic approach is envisaged involving all actors of human and veterinary medicine, animal husbandry, agriculture, environment, and legislation

7.2 UPDATE ON TRADITIONAL AND EMERGING BIOLOGICAL HAZARDS IN POULTRY AND POULTRY MEAT

Poultry and poultry meat might harbor food-borne bacterial pathogens. Contamination of broilers and meat by the two most prevalent gastrointestinal bacterial pathogens in humans in the EU, *Salmonella* and *Campylobacter*, are traditionally reported each year in the Zoonoses Summary Reports of European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) (EFSA and ECDC, 2015a).

Within the framework of Regulation (EC) No 2160/2003 on the control of zoonoses, National Control Programmes were implemented and permanent targets (<1%) were set in the European Union for the reduction of *Salmonella enteritidis* and *Salmonella typhimurium* (including monophasic *S. typhimurium* with the antigenic formula 1,4,[5],12:i) in broilers of *Gallus gallus* and in breeding and fattening turkeys (Reg. (EC) No. 200/2012; Reg. (EC) No. 1190/2012). As a consequence over the years 2011−14, there was an overall decrease in the proportion of broiler and turkey flocks positive for *Salmonella*-targeted serovars with a prevalence of 0.2% in 2014. A decreasing trend was observed also in fresh poultry meat for which a microbiological criterion was agreed (Reg. (EC) No 1086/2011). In 2014, only 0.1% of single samples and 0.2% of batches of fresh poultry meat were noncompliant in Europe (EFSA and ECDC, 2015a). Unfortunately, this decreasing trend was not reflected in the percentage of strong-evidence outbreaks caused by *Salmonella enterica* serovar Enteritidis or Typhimurium associated with the consumption of contaminated broiler meat and products thereof (1.6% in 2011 to 2.8% in 2014 with a pick of 5.3% in 2013) (EFSA and ECDC, 2015a; EFSA and ECDC, 2015b; EFSA and ECDC, 2014; EFSA and ECDC, 2013). These data might be linked to the observation that, although the *Salmonella* targets in poultry had an effect on poultry primary production and fresh poultry meat, the same seems not to be true in the later stages of the food chain were poultry meat is processed. No decreasing trends were registered in the same period in minced poultry meat and meat preparations to be cooked before consumption with proportions of noncompliant units around 8% (EFSA and ECDC, 2015a). Besides *S. enteritidis* and *S. typhimurium* (including the monophasic variant), other serovars might be considered in the future. According to risk-assessment studies, *S. Infantis* was estimated to be the second most prevalent causative agent, after *S. enteritidis*, of all broiler-associated human salmonellosis cases, with an estimated prevalence of 23%. Similarly, *S. kentucky* was estimated to be the second most prevalent causative agent after *S. enteritidis*, of the

turkey-associated human salmonellosis cases with an estimated prevalence of 17% (Messens et al., 2013).

Campylobacter might colonize the gut of chickens to contamination levels frequently higher than 10^6 colony-forming units per gram (CFU/g) of cecal content without inducing pathological signs in birds (Hermans et al., 2012). At slaughterhouses, broiler carcasses might become contaminated by *Campylobacter*-positive cecal contents during evisceration. In humans, contaminated broiler meat is considered to be the main source of human campylobacteriosis. Undercooking and cross-contamination in household kitchens are two risk factors accounting for 20%–30% of human cases (BIOHAZ, CONTAM and AHAW Panels, 2012). Significant percentages of *Campylobacter*-positive broilers and broiler meat were registered in 2014. Contaminated broilers were found with a mean percentage per EU Country of 28.8% with picks over 70% in some EU Countries (Greece, Portugal, and UK) (EFSA and ECDC, 2015a). Besides the prevalence, the level of contamination was found to be high in 22% of positive broilers tested from July to September 2014 (more than 1000 CFU/g). Regarding fresh broiler meat, in 2014 in Europe 38.4% of the samples (single or batch) at every sampling stage were found to be *Campylobacter*-positive, which was comparable to that in 2013 (EFSA and ECDC, 2015a).

In order to face the *Campylobacter* challenge, realistic targets should be agreed and specific control strategies should be implemented to achieve those targets. Since 2008, different countries worldwide implemented or agreed with industries on specific *Campylobacter* targets and/or *Campylobacter* criteria. Targets for reduction at either animal or food level were agreed in New Zealand, UK, Ireland and USA (MPI, 2015; FSA, 2010; FSAI, 2011; FSIS, 2016). In order to reach those targets of reduction, specific control strategies both at animal and food-chain level have been studied and implemented in National Action plans (i.e., in Norway, UK, USA) (Pasquali et al., 2011; Keener et al., 2004). Details on specific control strategies are reported in the following paragraphs.

Besides traditional bacterial pathogens, in recent years special focus has been addressed to *Arcobacter* and *H. pullorum* as emerging biological hazards in poultry meat. *Arcobacter* is part of the family *Campylobacteraceae*. As for *Campylobacter*, *Arcobacter* is found responsible for human gastrointestinal infection often through the consumption of contaminated poultry meat. In the past, the similarity between the two genera has been probably responsible for the potential misidentification of *Arcobacter* leading to an underestimation of the distribution and prevalence of this genus. Among the genus *Arcobacter*, four species were identified as relevant for humans: *Arcobacter butzleri, Arcobacter skirrowii, Arcobacter cryaerophilus*, and the recently reported *Arcobacter thereius* (Vandamme et al., 1992; Van den Abeele et al., 2014; Ferreira et al., 2015).

Arcobacter spp. is generally not found in live birds and their immediate environment. However, *Arcobacter* was found in poultry slaughterhouses, suggesting that poultry carcasses are contaminated during processing (Gude et al., 2005; Aydin et al., 2007). Variations in the percentage of positive samples are observed around the world at poultry slaughterhouses ranging from approx. 30% (Germany and Belgium) to 96%–100% Denmark and US) (Houf et al., 2003; Atabay et al., 2006; Atanassova et al., 2008). High percentages of *Arcobacter* (around 55%) have been identified also in the following steps of the food chain, although a significant decrease in the occurrence during processing was observed up to an overall occurrence of 10%–30% at retail level (Son et al., 2007; Rahimi et al., 2012; Villalobos et al., 2013; Rahimi, 2014; Mohan et al., 2014; Bagalakote et al., 2014). Exceptions were found in Australia, Spain, and Northern Ireland with higher occurrence rates in chicken meat of 73%, 64.3%, and 62%, respectively, although a relatively low number of chicken meat samples were tested in all the three studies (Rivas et al., 2004; Scullion et al., 2006; Collado et al., 2009). For a comprehensive view on the distribution and prevalence of *Arcobacter* around the world at the different steps of the food chain, please refer to the previously published review by Hsu and Lee (2015).

H. pullorum is an enterohepatic helicobacter species colonizing the GIT and the biliary tree of the host. *H. pullorum* pathogenicity of some strains have been linked to the production of a cytolethal distending toxin (CDT) with a cytopathogenic effect on epithelial cells (Young et al., 2000; Varon et al., 2014; Borges et al., 2015). As for *Campylobacter* and *Arcobacter*, *H. pullorum* belongs to the order of *Campylobacteriales* although to the distinct family of *Helicobacteriaceae*. As for *Arcobacter*, due to the strong similarity of *H. pullorum* with *Campylobacter* and in particular to *C. coli*, the identification of this species might have been underestimated in the past (Melito et al., 2000).

Regarding the sources of isolation, *H. pullorum* was isolated from the feces and liver of poultry and from healthy and diseased humans with gastroenteritis (Stanley et al., 1994; Burnens et al., 1994; Ceelen et al., 2005). As poultry carcasses and fresh meat can be contaminated by *H. pullorum*, this bacterium can be considered as a potential emerging foodborne pathogen (Atabay et al., 1998; Borges et al., 2015). Studies reported prevalence percentages of around 4% in both healthy humans and patients with gastroenteritis in Belgium (Ceelen et al., 2005). In poultry, occurrences of 76.4% and 81.8% in ceca samples of turkeys and broilers, respectively, were described in Italy (Zanoni et al., 2011; Manfreda et al., 2011). No data are available on the prevalence of *H. pullorum* in poultry meat. Besides prevalence, also the virulence, colonization, and adaptation features of this bacterium are not fully clarified, although a boost has been recently achieved with metagenomics and whole-genome-sequence studies (Kaakoush et al., 2014; Borges et al., 2015).

7.3 CHALLENGES TARGETING LIVE POULTRY

7.3.1 Application of the Metagenomic Approach to Promote the Gut Health

Health and nutrition are interdependent in chickens as well as in humans. The interaction between the two factors occurs largely in the GIT and is mediated by a complex and largely unknown microbial community representing the GIT microbiota. Such a community includes commensal, symbiotic, and pathogenic organisms, which can be bacteria, viruses, parasites, etc. The microbiota is a dynamic entity and provides the host with an extensive array of enzymes and substrates, resulting in products that contribute to host energy and nutrient supply, immunity and general well-being (Brisbin et al., 2008; Stanley et al., 2014a; Choi et al., 2015). For bacterial taxa inhabiting the poultry GIT, a limited part has been recovered by cultivation, mainly due to their requirement for anaerobic conditions, similar to those in the GIT. Therefore, in order to predict nutritional and ecological roles of GIT microbiota, researchers have turned to new culture-independent approaches, and metagenomics is one of those. Metagenomics refers to a nonculture-based approach for collectively studying sets of genomes from the microbiota (Handelsman, 2004).

High-throughput next-generation sequencing (NGS) technologies can be utilized for metagenomic analysis through the generation of large volumes of sequence data containing genetic information (Metzker, 2010).

The goals for metagenomic analysis are to understand: (1) community composition/structure, including the taxonomic breakdown and relative abundance of the various species; (2) genic contribution of each member of the community, including number and functional capacity; (3) intra-species or intra-population heterogeneity of the genes (Scholz et al., 2012).

Metagenomics provides significant information on the changes and shifts in the microbial community of the chicken GIT, impacting on host performance and health. The microbial communities colonizing the GIT differ among areas and have specialized digestive functions. A detailed overview of chicken indigenous bacteria for each GIT section is given in the reviews by Rehman et al. (2007) and Yeoman et al. (2012) (Fig. 7.1). Within the GIT, the ceca are an important site of fermentation (Mead, 1989; Clench and Mathias, 1995), influencing animal health and performance; therefore the cecal microbiota profiles are widely investigated. The most abundant groups in the chicken ceca were found to be Clostridiaceae, Bacteroidaceae, Lactobacillus, Proteobacteria, butyrate-producing cluster, and unknown Firmicutes, with an abundance of Clostridium, Ruminococcus, Eubacterium, Faecalibacterium, and Lactobacillus species among a number of unknown and uncultured phylotypes. The genes associated with carbohydrate metabolism are consistently abundantly represented in metagenomic libraries (Qu et al., 2008; Danzeisen et al., 2011; Sergeant et al., 2014).

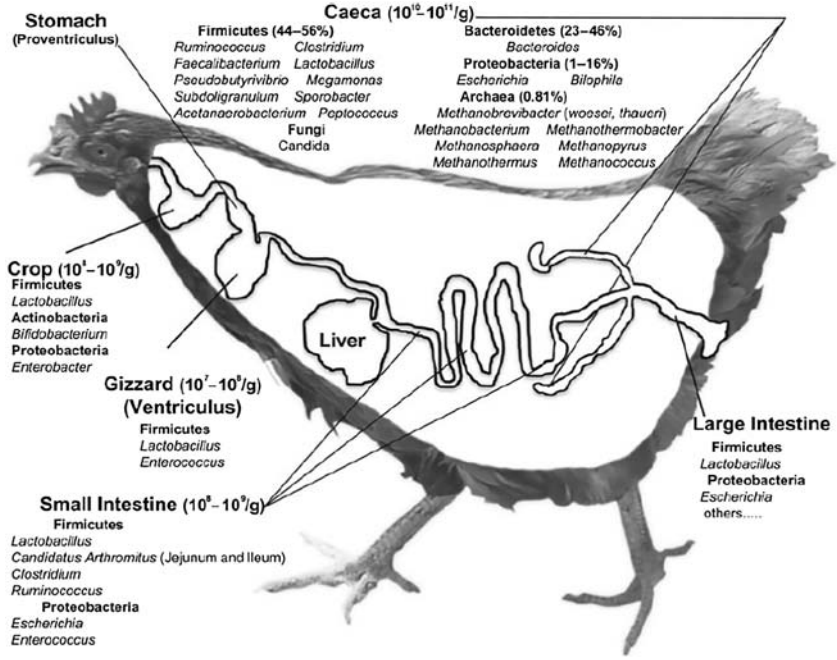


FIGURE 7.1 Dominant microbial taxa along the chicken gastrointestinal tract (Yeoman et al., 2012).

The gut microbiota is one of the main defence components in the GIT against enteric pathogens (Pourabedin and Zhao, 2015). Disturbance of the gut microbiota–host interaction plays a crucial role in the development of intestinal disorders (Pourabedin and Zhao, 2015). Significant changes in cecal microbiota have been evident in chickens infected with *Clostridium perfringens* (Feng et al., 2010; Stanley et al., 2012; Skraban et al., 2013), *Eimeria* species (Perez et al., 2011; Stanley et al., 2014b; Wu et al., 2014) and *S. enteritidis* (Nordentoft et al., 2011; Juricova et al., 2013; Videnska et al., 2013). The chicken gut microbiota has also modulated intestinal gene expression (Yin et al., 2010), T cell-mediated immunity (Mwangi et al., 2010), and accelerated gut immune system maturation (Crhanova et al., 2011). Furthermore, microorganisms in the gut interact with each other as well as with the host, influencing many physiological functions within the host. Several bacterial phylotypes, more specifically within genera *Lactobacillus*, *Ruminococcus,* and *Clostridium*, were associated with performance enhancement (Torok et al., 2008; Stanley et al., 2013).

Normal changes in the microbiota occur over time and can be related to factors such as age (Hanning and Diaz-Sanchez, 2015). For example, the chicken GIT is sterile at hatching but is quickly colonized by aerobic

Proteobacteria and after 12 days is dominated by anaerobic Firmicutes. Initially, the Proteobacteria stimulate the histological maturation of the GIT and provide an ideal environment for the Firmicutes. The Proteobacteria do well in the immature GIT but are poor competitors and are outcompeted after a mature and anaerobic gut environment is established. The succession of bacteria is also dependent on nutrition and gut bacteria population selection, and establishment can be driven by feeding specific molecules (Hanning and Diaz-Sanchez, 2015). This strategy can be followed also in order to achieve an accelerated growth rate. In fact, growth promotion can be obtained by delivering probiotic cultures to animals and hastens the histological development of the GIT (Patterson and Burkholder, 2003). The mature GIT improves nutrient uptake and increases the growth rate of the animal (Patterson and Burkholder, 2003). It has been argued that the effect of improved growth rate in probiotic-treated animals is temporal, and non-treated counterparts eventually meet the same weight (Higgins et al., 2002).

In the context of chicken health, an application of metagenomic analysis includes determining and resolving resistance of infectious pathogens through the prediction of susceptibility to disease and drug response and, for instance, the early detection and molecular classification of diseases. The release of the first draft of the chicken genome sequence in 2004 (International Chicken Genome Sequencing Consortium, 2004) has provided almost 7 million single nucleotide polymorphism (SNP) (Rubin et al., 2010). Gut microbial flora/communities of poultry can now be sequenced rapidly to determine any changes that affect health and disease and to provide a detailed assessment of probiotics or prebiotic methods to control pathogenic growth. In addition, function-based metagenomic analysis can be used as a screening tool for antibiotic resistance patterns and vitamin production within microbial communities (Diaz-Sanchez et al., 2013). The application of metagenomic techniques in poultry production could lead to the development of novel alternatives to antibiotic growth promoters and comparative genome analysis (i.e., mechanisms of *S. enterica* evolution and transcriptomics of *S. enterica* plasmids (Johnson et al., 2012; Lin et al., 2012)). Also, through the application of metagenomics, it has been possible to obtain complete sequences of a new bacteriophage with antimicrobial properties (Avant, 2012).

Metagenomic analyses in combination with other approaches will allow the identification of the real players among GIT microbiota that help in host metabolism and in maintaining its health. Moreover, metagenomic investigations can provide a wide view toward the control of diseases in poultry as well as research related to enteric foodborne pathogens (Frank and Pace, 2008). Among them, *Campylobacter* must be mentioned because it causes a large part of foodborne zoonoses in the EU and poultry is one of the main sources of this pathogen (EFSA and ECDC, 2015a). The development of whole-genome sequencing of *Campylobacter jejuni* offered the opportunity of detection of genes and proteins involved in the pathogenesis of

Campylobacter and also genes of unknown function (Parkhill et al., 2000; Stahl and Stintzi, 2011). Perhaps the understanding of the function of many of these proteins and genes could lead to the improvement of current practices and potential intervention strategies regarding the role of *Campylobacter* in the chicken gut, colonization, and immune response (Diaz-Sanchez et al., 2013).

7.3.2 The Challenge of Reduction of Antimicrobial Resistance in Poultry Production

At present, the significant increase of AMR bacterial pathogens in humans raises a serious concern worldwide due to important treatment failures against microbial infectious diseases. Four-million patients are estimated to acquire healthcare-associated infections every year in Europe, with 25,000 human deaths due to AMR pathogens (EC, 2011).

These observations prompted the European Commission to implement specific strategies toward a prudent use of antimicrobial agents in human medicine and a containment of the increase of resistant pathogens. These strategies should comprise measures in relation to surveillance, education, information, prevention and control, and research (2002/77/EC). In 2011, the EU commission defined an Action Plan against the rising threats from AMR (EC, 2011). In this Action Plan, a holistic approach was envisaged involving all actors of human and veterinary medicine, animal husbandry, agriculture, environment, and trade. The overall aims of this action plan were: (1) to ensure an appropriate use of antimicrobial drugs both in human and veterinary medicine in order to mitigate the risk of AMR developing in humans by promoting microbial diagnosis; (2) to implement effective strategies to prevent microbial infections and their spread; (3) to develop effective antimicrobials or alternatives for treatment; (4) to join forces with international partners to contain the risks of spreading AMR from country to country through traveling and the environment; (5) to reinforce research on innovative measures against AMR (EC, 2011). Different EU Countries implemented National Action Plans for a more prudent and rational use of antimicrobials (i.e., France, UK, Germany, and The Netherlands). Recently the EU Commission published (2015/C 299/04) practical guidelines on measures to be adopted in general and in each specific food-producing-animal species with the overall goal of preventing infectious diseases by improving health, welfare, and immunity. Additionally, the EU recommends avoiding or restricting the metaphylactic and prophylactic use of antimicrobials, as well as the use of critical important antimicrobials in veterinary medicine.

In poultry, specific requirements were reported for the implementation of welfare programs (i.e. footpad scores), avoidance of antimicrobials in eggs or day-old-chicks in hatcheries as well as at the farm upon the arrival of 1-day-old chicks, the avoidance of third- and fourth-generation cephalosporins and restriction of fluoroquinolones (2015/C 299/04).

Along with these requirements, specific strategies have been implemented or are currently under study in poultry and specifically in broilers as preventive measures to reduce the likelihood of infectious diseases and therefore the use of antimicrobials. These measures can be classified into four groups: biosecurity, vaccination, feed additives as alternative to antimicrobials, and disease resistance breeding (Table 7.1).

Biosecurity includes all actions aimed at preventing the introduction of a pathogen into a flock (external biosecurity) and at preventing the spread of the pathogen between flocks (internal biosecurity). In poultry farms, in order to prevent the horizontal transmission of pathogens from the external environment, special attention should be paid to feed, water, visitors and personnel, wild animals and pets (Heyndrickx et al., 2002; Newell et al., 2011; Pasquali et al., 2011; OIE, 2015). Along with horizontal spread, vertical transmission routes from breeders to eggs are also to be considered for specific pathogens like *Salmonella* and *Mycoplasma* (Cobb, 2011). In this frame, the quality of the 1-day-old chicks as well as their transport (duration-temperature, hygienic status of transport containers, etc.) are essential aspects to be monitored (Cobb, 2011). Removal of manure and dead animals is also important. Despite the fact that all these external and internal biosecurity measures are well known, their implementation at farm level is often poor; therefore, the education of personnel should be encouraged. Another essential point for improvement is holding design and building (OIE, 2015). Specific engineering studies should be performed in collaboration with veterinarians, epidemiologists, and microbiologists to optimize ventilation and reduce dust inside the poultry house in order to reduce the disease transmission challenge both between rooms and between buildings. Finally, tools should be available to measure and quantify the level of biosecurity in broiler farms. As an example, a biosecurity risk-weighted score system was designed to help farmers to evaluate the level of biosecurity in their farms and to compare their scores with national averages (Gelaude et al., 2014).

Along with good biosecurity, *vaccination* can be essential to an effective on-farm control program. Vaccines against bacterial infectious diseases can be based on live (attenuated) organisms or killed (inactivated) organisms, or based on virulence proteins (inactivated or not inactivated toxins) often administered in combination with highly antigenic proteins (Van Immerseel et al., 2005; Shil et al., 2011; Mot et al., 2014). Live attenuated vaccines have been shown to be more effective than inactivated vaccines since they both induce a cell-mediated and an immune response (Van Immerseel et al., 2005). Effective live attenuated vaccines were described against *Mycoplasma gallisepticum* and *Salmonella* (Van Immerseel et al., 2005; Shil et al., 2011). Although effective, live vaccines raise safety issues due to their variable pathogenicity and genetic manipulation. Moreover, their induced protection can be extremely variable due to the dose, the level of colonization and

TABLE 7.1 Current Preventive and Alternative Strategies to Control the Prevalence and Spread of Bacterial Pathogens and Reduce the Use of Antimicrobial Drugs in Broilers and Broiler Meat

Strategy	Focus	Mode of Action	Efficacy
Broilers			
External and internal biosecurity	Quality of feed, water and chicks; good hygiene practices for workers; holding design (ventilation, fly screens)	Control the horizontal and vertical transmission of bacteria from the external environment to the interior of the poultry farm and from bird to bird	Very high especially for bacteria of environmental origin such as campylobacteriales
Vaccination	Live or inactivated vaccines	Stimulate cell-mediated and/or humoral immunity	Very high but narrowed to the bacterial pathogen. Safety concerns on live vaccines
Feed additives: pre- and pro-biotics	MOS (mannan-oligosaccharides) and FOS (fructo-oligosaccharides); Lactobacilli, Bifidobacteria, and other bacteria stimulating health	Reduce pathogens shedding; stimulate immunity of the host and growth of beneficial bacteria in the gut	Moderate. To be coupled with other strategies
Feed additives: essential oils	Thymol	Antimicrobial properties	Moderate. To be coupled with other strategies
Feed additives: organic acids	Butyric acid	Reduce pathogen shedding; stimulate epithelial cell proliferation and differentiation, harbor antimicrobial properties	Moderate. To be coupled with other strategies
Feed additives: amino acids	Methionine	Stimulate immunity	Moderate. To be coupled with other strategies

(*Continued*)

TABLE 7.1 (Continued)

Strategy	Focus	Mode of Action	Efficacy
Feed additives: clay minerals	Mycotoxin binders	Detoxifying properties by binding toxic compounds such as mycotoxins	Relevant in case of mycotoxin contaminated feed
Bacteriophages and bacteriocins		Antimicrobial properties	Against *Campylobacter*
Metagenomics and genetic selection	Gut microbiota population abundances and identification of genetic markers of poultry disease resistance	Assessment of microbial gut composition and improvement of immunity response to pathogen bacteria	Potentially high
Broiler meat			
Physical carcass treatments: freezing	Freezing followed by frozen storage of up to 31 days	Reduction of *Campylobacter* load up to 2.87 $\log_{10}$ CFU/kg of carcass	Overall 10% reduction in *Campylobacter* prevalence in broiler meat
Physical carcass treatments: chilling	Air chilling (AC) and water immersed chilling (WIC)	WIC was linked to a higher *Campylobacter* load reduction then AC	No effect on overall *Campylobacter* prevalence on broiler carcasses
Physical carcass treatments: steam	Treatment for 10–20 s alone or coupled with ultrasound	Reduction of up to 3.3 $\log_{10}$ CFU/cm^2 of carcass	Possible side effects on meat quality
Physical carcass treatments: irradiation	Electron beam irradiation at 1.0 kGy	Eradication of *Campylobacter* from chicken breast	Very high. Side effects on meat quality
Chemical treatments	Peracetic acid, hydrogen peroxide	Antimicrobial properties	To be confirmed. Not approved in the EU for poultry carcass decontamination

(*Continued*)

TABLE 7.1 (Continued)

Strategy	Focus	Mode of Action	Efficacy
Management	Slaughterhouse	Differential management of heavily *Campylobacter*-contaminated broiler carcasses	High
Risk management	Broiler food chain	Identification of risk based metrics such as Performance Objectives (PO) and Food Safety Objectives (FSO)	High. Requires a great interaction between scientists, policymakers, and food operators

persistence of the live vaccine strain, the level of expression of antigens, the severity of the challenge (Mot et al., 2014; Shil et al., 2011). Regarding protein-based vaccines, Net B or Alpha toxin alone was not shown to induce a significant protection against *C. perfringens*. However a combination of bacterin (50:50 (v/v) bacterial cells and culture supernatant), crude toxoid (formaldehyde-treated culture supernatant) and rNetB (purified NetB recombinant protein) was able to significantly protect birds against a moderate and severe in-feed challenge after subcutaneous vaccination at days 7 and 17 (Keyburn et al., 2013). Besides the different types of vaccines, the way vaccines are delivered is an issue which is extremely important for their successful use in poultry production. Often in research studies, vaccines are delivered by parenteral administration (Groves et al., 2015; Mot et al., 2014). This type of delivery does not fit with the management practices of a broiler industry. Ideally, a vaccine should be delivered by feed or drinking water or spray (Mot et al., 2014).

According to Regulation (EC) No 1831/2003, *feed additives* are substances, molecules, or microorganisms, intentionally added to feed or water in order to favorably influence animal production, performance or welfare, particularly by affecting the gastrointestinal flora or digestibility of feedstuffs. Among these, several substances were shown to enhance the health and immunity status of animals and/or to have antimicrobial activities and were therefore defined as alternatives to antimicrobials (Cheng et al., 2014). Feeding enzymes, essential oils, organic acids, probiotics, amino acids, and clay minerals have been evaluated as alternatives. An increase in indigestible water-soluble nonstarch polysaccharides has been linked to increased proliferation of pathogen bacteria such as *C. perfringens* predisposing birds to necrotic enteritis (M'Sadeq et al., 2015). Essential oils were

shown to improve nutrient digestibility and to harbor beneficial antibacterial properties against *E. coli, C. perfringens*, and *Salmonella*. However unwanted antimicrobial activities against beneficial bacteria such as *Lactobacilli* and *Faecalibacterium prausnitzii* in healthy gut microbiota of chickens was also observed (Zeng et al., 2015). Organic acids such as butyric acid was also shown to have antimicrobial activity along with functions of protection against pathogens and stimulation of the epithelial cell proliferation and differentiation (Van Immerseel et al., 2006; Dalmasso et al., 2008; Fernandez-Rubio et al., 2009). Prebiotics and probiotics are substances and live microorganisms, respectively, which confer a health benefit to the host by increasing beneficial bacterial populations such as Bifidobacteria and Lactobacilli and controlling pathogen bacteria. MOS (mannan-oligosaccharide) and FOS (fructose-oligosaccharides) were described to reduce (up to one $\log_{10}$ CFU/g) *Clostridium difficile* and *E. coli* population at the ileal/ceca junction of 4-week-old broilers (Kim et al., 2011). Combinations of microorganisms such as undefined or partially defined cultures derived from poultry intestinal microbiota were used in the past as probiotics showing positive protective effects by competitive exclusion (Nakamura et al., 2002; Kabir, 2009). Unfortunately, due to a lack of a full characterization of single microorganisms within the probiotic, these feed additives are currently facing problems for the renewal of authorization. The administration of probiotic Lactobacilli has been demonstrated to stimulate immune responses (Haghighi et al., 2005; Brisbin et al., 2011), improve digestive health (Kim et al., 2012), as well as growth performance (Loh et al., 2010; Shim et al., 2012; Askelson et al., 2014) in poultry. Lactobacillus administration has also been shown to reduce colonization by *Campylobacter* (Ghareeb et al., 2012; Neal-McKinney et al., 2012), *Clostridium* (La Ragione et al., 2004), and *Salmonella* (Chen et al., 2012; Ghareeb et al., 2012). Other immunostimulatory feed additives tested in poultry are chitosan and specific amino acids such as methionine (Swiatkiewicz et al., 2015; Jankowski et al., 2014). For a comprehensive review on immunostimulators as feed additives, please refer to the EFSA recent publication (IRTA, 2015). Clay minerals have detoxifying properties. By binding toxic compounds, clay minerals reduce their toxicity and bioavailability. Their effect is particularly relevant against micotoxins which might be present in feed and may induce acute and long-term chronic effects or immunosuppression (Huwig et al., 2001).

In recent decades, breeding for *disease resistance* has gained more attention especially in relation to the development of the -omics approaches. The selection of birds with anti-disease ability or reduced susceptibility to a particular disease might be: (1) direct selection: breeding chickens are challenged and the resistant birds are selected; (2) indirect selection: birds are selected for particular resistant indicators such as genes, pathogen products, and biological or immunological responses of the host; (3) transgenosis: viral

vectors are used to produce transgenic disease-resistant birds; (4) genomic selection: the genomes of chickens are studied in order to identify high heritable genetic markers for disease resistance and these markers are used to predict the disease-resistant traits of chicks by calculating a genomic estimated breeding value (GEBV) (Jie and Liu, 2011). Genomic selection gained particular interest toward the improvement of antibody response to Newcastle Disease, Avian Influenza Virus and Marek's Disease (Cheng et al., 2013; Luo et al., 2013; Liu et al., 2014; Cheng et al., 2015). Studies on the heritable basis of host resistance were also performed against *Salmonella* and *Campylobacter* (Cheng et al., 2013).

7.3.3 The Challenge to Control *Campylobacter* in Live Poultry

Campylobacter is the most commonly reported cause of human intestinal infections in the world. At the European level, more than 200,000 confirmed cases of campylobacteriosis were reported in 2014. Several strategies have been investigated to reduce the shedding of *Campylobacter* in primary production since this stage of the food chain influences the quality of the subsequent stages and therefore the impact on public health (Chemaly, 2015; Meunier et al., 2015a).

One of the possible measures is to strengthen biosecurity at the farm level in order to limit the introduction of *Campylobacter* in the environment of poultry and prevent colonization of birds. Many studies have shown a reduction in colonization by applying good hygiene practices such as the use of overshoes, disinfecting footbaths, changing shoes between holdings, and disinfection of trucks (Gibbens et al., 2001; Rivoal et al., 2005; Newell et al., 2011; Ridley et al., 2011). In Denmark, the use of fly screens to prevent the introduction of flies to farms has proved an effective measure to decrease the prevalence from 51% to 15% (Hald et al., 2007). If these studies have shown an effectiveness in some farms, their implementation in all farms remains difficult because they are costly and not adapted to all farming practices such as those encountered for some avian production requiring access to a free range.

Another possibility of intervention in primary production to control shedding of the bacteria since the ban on antibiotics as growth promoters is the supplementation of feed additives having an inhibitory action against *Campylobacter*. Large categories of products have been tested including organic and fatty acids (Chemaly et al., 2007a; Solis de Los Santos et al., 2008; Hermans et al., 2010; Jansen et al., 2014; Guyard-Nicodème et al., 2016), pre- and probiotics (Santini et al., 2010; Ghareeb et al., 2012), plant extracts and essential oils (Hermans et al., 2011; Robyn et al., 2013; Guyard-Nicodème et al., 2016), bacteriophages and bacteriocins (Wagenaar et al., 2005; Stern et al., 2006; Carvalho et al., 2010; Connerton et al., 2011; Messaoudi et al., 2012; Fischer et al., 2013). Other products derived from

whey (Chemaly et al., 2007b) and clay (Guyard et al., 2013) were also tested as alternatives for *Campylobacter* reduction in live animals. Despite the diversity of published results about the effectiveness of products to reduce shedding of *Campylobacter*, all agreed that no product allowed the total elimination of *Campylobacter* in live birds. The effectiveness was highly dependent on products, avian strain (Guyard et al., 2014; Humphrey et al., 2014) and application conditions. A large collaborative EU project "Campybro" aimed at developing different control measures at the farm level through two strategies, administration of additives in feed or animal vaccination, has been running since 2013. The main results based on additives belonging to four families, organic and fatty acids, plant extracts, prebiotics and probiotics, showed possible reductions at the farm level using additives in feed (Guyard-Nicodème et al., 2016: Gracia et al., 2016); significant decreases were observed at least at one of the sampling points between 14 and 42 days after infection. One additive, a butyrate-based product, presented a significant effect throughout the study from 14 to 42 days with a reduction of about 1.5 $\log_{10}$. On the other hand, a probiotic and a prebiotic-based product allowed a reduction of 1 and 2.5 $\log_{10}$, respectively, but only at 42 days. These results suggested that some additives could be distributed from the beginning of farming period as a preventive action before slaughtering the animals, while others could be distributed some days before slaughtering as a curative action (Guyard-Nicodème et al., 2016).

In general, the strategy of administration of additives in feed seems inadequate on its own to reduce *Campylobacter* loads in live animals in a significant and reproducible way. It should be coupled to another strategy such as biosecurity measures at the primary production level but also to other strategies at the following steps of the broiler chain, processing, retail and at the consumer level to achieve a reduction able to impact significantly the decrease of campylobacteriosis.

Although all the studies reported are still being conducted, vaccination seems to be a promising strategy to control *Campylobacter* (Meunier et al., 2016a). Several vaccination trials were carried out either with whole cells of *C. jejuni* inactivated vaccine (Rice et al., 1997; Ziprin et al., 2002), the subunit vaccines such as the flagellin (Lee et al., 1999; Huang et al., 2010) or other membrane proteins (Annamalai et al., 2013), antigens carried by microorganisms (Wyszynska et al., 2004; Laniewski et al., 2014). The results of these studies were heterogeneous for reasons related first to the absence/partial of protection during in vivo trials, the difficulty to reproduce, and to the lack of control of the immune system of animals. Within the EU project "Campybro", researchers are working on a new vaccination strategy based on the identification of new antigens and evaluation of their potential to protect animals from *Campylobacter* colonization (Meunier et al., 2015b; 2016b). This promising tool called "reverse vaccinology" (Rappuoli, 2000) requires the use of powerful genomic technologies to

identify novel antigens using bioinformatics and sequencing. Following this method, 14 proteins, not described previously, were identified as potential vaccine antigens (Meunier et al., 2016b). Testing these in vivo would bring new insights into the vaccination strategy in poultry.

7.4 CHALLENGES TARGETING POULTRY MEAT

7.4.1 Control Strategies for *Campylobacter* at the Level of Meat Processing

During slaughter processing, the contamination of carcasses by *Campylobacter* occurs generally by a cross-contamination with the ceca of infected birds. The correlation between the cecal load and carcass contamination is very well established (Reich et al., 2008; Hue et al., 2011). Cross-contamination between negative birds and positive ones may also occur and this can be explained by the sharing of the same lines of production independently of the birds' *Campylobacter* status and instrumental contamination from infected animals. It has been shown that visceral rupture during the evisceration operation leads to an increase in *Campylobacter* of 0.9 $\log_{10}$ units per carcass (Boysen and Rosenquist, 2009). In order to decrease *Campylobacter* contamination on carcasses at slaughter, several treatments are discussed in the section below and summarized in Table 7.1, although at the EU level no treatment is actually performed.

7.4.1.1 Physical Treatments

Several studies investigated the beneficial effects of carcass freezing on the decrease of *Campylobacter* loads. The one conducted in Iceland showed significant reductions on *Campylobacter* counts when chicken carcasses were frozen and stored at $-20^{\circ}C$ for 31–220 days compared to fresh products. However, *Campylobacter* levels remained quite constant between the different storage periods indicating no beneficial impact of the storage duration (Georgsson et al., 2006). Tested on different flocks, freezing followed by a frozen storage of 31 days led to significant *Campylobacter* reductions (from 0.65 to 2.87 $\log_{10}$ CFU/kg of carcass). Prior to freezing and storage, all samples were contaminated at levels exceeding 10^4 CFU/kg of carcass, whereas after freezing and storage, 90% of the samples had less than 10^4 CFU/kg of carcass. On the other hand, compared to freezing, cold storage at $+3^{\circ}C$ did not significantly reduce *Campylobacter* loads during 7 days of experimentation. Crust freezing using CO_2 appeared to be less efficient than common freezing. In fact, this treatment allowed a reduction of 0.42 $\log_{10}$ CFU per carcass comparing to 1 $\log_{10}$ reduction obtained after classical freezing (Boysen and Rosenquist, 2009).

One of the processing measures implemented in Denmark was to channel *Campylobacter*-negative flocks to chilled meat production and

Campylobacter-positive flocks to frozen production because of the bacterial load reduction after freezing (Rosenquist et al., 2009). This allowed an annual prevalence reduction by about 10% between 2004 and 2007. Nevertheless, human campylobacteriosis incidence did not decrease along with the study, which could be explained by the increase of imported contaminated products and meat from noncontrolled slaughterhouses available in retail.

Air-chilled (AC) and water-immersion-chilled (WIC) treatments were also evaluated regarding *Campylobacter* reduction on carcasses. The results indicated that WIC was significantly more effective at reducing *Campylobacter* load than AC. However, prevalence data showed no significant reduction of *Campylobacter* on broiler carcasses between the two chilling methods and compared to the post-evisceration prevalence (Zhang et al., 2011). Several other studies reported the best efficiency of WIC compared to AC such as the one of Berrang et al. (2008), who demonstrated lower *Campylobacter* levels from half-broiler carcasses after immersion chilling compared to carcasses after air-chilling treatment.

The optimization of chilling parameters appeared to be an optimal strategy to contribute to *Campylobacter* reduction on carcasses since this pathogen is sensitive to cold temperature. In a study conducted on a chilling pilot usable at the laboratory level, four parameters were selected to assess their impact on *Campylobacter* counts on broiler legs artificially contaminated: *C. jejuni* initial load, chilling temperature, duration, and air flow (Rivoal et al., 2014). Significant parameters were the initial load of *C. jejuni* ($P = 0.03$) and duration ($P = 0.04$). However, the most relevant result indicated that *Campylobacter* load on carcasses is the most significant parameter and heavily contaminated carcasses ($>10^3$ CFU/g) cannot be significantly decontaminated after chilling (Rivoal et al., 2014; 2015).

Carcasses inoculated experimentally and submitted to steam for 10, 12, or 20 s showed significantly lower *C. jejuni* AR6 counts and the treatment of 20 s duration was likely to be more effective with greater reduction (3.30 $\log_{10}$ CFU/cm^2) compared to 10- and 12-s steam application. However, such treatment resulted in skin shrinkage and color changes (James et al., 2007). Moreover, the authors reported that reductions obtained with steam or hot water were greater when combined to chilling at 0°C or crust freezing. A recent study described the combination of steam and ultrasound (SonoSteam) as broiler carcasses' treatment (Musavian et al., 2014). Breast skin samples were excised from naturally contaminated birds before and after SonoSteam treatment and *Campylobacter* load was determined. A significant 1 $\log_{10}$ reduction was observed after the treatment. No alteration of meat quality was detected compared to untreated carcasses. This technique was used in a previous study and showed a significant *Campylobacter* load reduction of more than 2.5 $\log_{10}$ CFU per carcass but with high variation between samples. Also, after the treatment, carcass skins appeared to be slightly boiled

(Boysen and Rosenquist, 2009). Some improvements of the technique could have been brought explaining the differences between the two studies.

7.4.1.2 Irradiation Treatments

Irradiation treatments have been evaluated regarding their capacity to decrease *Campylobacter* loads on chicken meat. Patterson (1995) used gamma ray irradiation (Colbalt 60) and showed D10 values, defined as the amount of radiation energy needed to decrease 90% of the target microorganism in irradiated food product, ranging from 0.12 to 0.25 kGy depending on *Campylobacter* species and strains. These values were lower than those obtained for *Salmonella* or *Listeria monocytogenes* under the same conditions involving a higher sensitivity of *Campylobacter* to radiation. Another study using electron beam irradiation at 1.0 and 1.8 kGy showed a total elimination of *Campylobacter* from chicken breast without any difference between the two doses which were under the authorized limit in the EU and the USA (Lewis et al., 2002). However, despite the effectiveness of radiation treatments, they are related to changes in meat quality such as color, lipid oxidation, flavor, or texture. In fact, Lewis et al. (2002) demonstrated that after 14 days of storage, differences in texture and flavor were observed for the 1.8-kGy radiations. Also, after storage for 28 days, the overall acceptability decreased for both irradiation dosages. To counteract the meat quality deterioration, a more recent study used irradiation associated with vacuum or with high-$CO_2 + CO$ modified atmosphere packaging (MAP) and showed that both types of packaging did not influence the effectiveness of irradiation at eliminating *Campylobacter* on chicken meat. A significant decrease in *Campylobacter* counts was observed with the increase of the dose radiation especially for the MAP packaging, whereas there was no difference between the two higher doses for the vacuum package. For the higher dose, a reduction by about 2.5−3 $\log_{10}$ CFU was reported. Moreover, the storage period from 1 to 6 weeks had no impact on *Campylobacter* counts in irradiated samples. However, a sour aroma and an off-odor from raw and irradiated chicken breasts were reported for both types of packaging (Kudra et al., 2012). Ultraviolet (UV) was also used for irradiation treatment on broiler meat, skin, and carcasses. Three UV doses were tested from 9.4 to 32.9 mW/s/cm^2. For broiler meat and skin, 0.7 and 0.8 $\log_{10}$ reductions in *Campylobacter* counts, respectively, were found. On broiler carcasses, UV irradiations alone or in combination with active oxygen, led to a 0.4 $\log_{10}$ reduction. No differences were observed in the color and the sensory quality between irradiated and nonirradiated samples. Due to the low *Campylobacter* reduction and the low human infective dose of *C. jejuni*, the authors did not recommend the use of UV irradiation as a primary measure of carcass decontamination, but suggested that it could improve the results when used in combination with other techniques (Isohanni and Lyhs, 2009).

7.4.1.3 Chemical and Biological Treatments

In the USA, chlorine is traditionally used as an antimicrobial in carcass chiller treatment but its efficacy is highly reduced by pH levels up to 7.0 and high organic loads. Several studies have investigated other antimicrobials such as hydrogen peroxide, chlorine dioxide, or organic acids. In 2008, Bauermeister et al. investigated peracetic acid in combination with hydrogen peroxide (PAHP) in poultry chillers. When treated with 85 ppm PAPH in a final water chiller and for approximately 20 min, *Campylobacter*-positive carcass numbers were reduced by 43% between prechill and postschill sampling points; whereas only a 13% reduction was obtained when treated with 30 ppm chlorine, suggesting a better antimicrobial activity of PAPH compared to chlorine (Bauermeister et al., 2008a). Researchers also investigated various peracetic acid (PAA) concentrations on carcasses experimentally inoculated with 10^6 *C. jejuni* (Bauermeister et al., 2008b). Only the chilled water treatment with the highest PAA concentration (0.02%) for 1 h led to a significant decrease in *C. jejuni* load by 1.5 $\log_{10}$ compared to the chlorine-treated group. Although, no changes in meat quality were observed 7 days after the treatment, samples from 0.015% and 0.02% PAA were the only ones that could be studied for the meat quality 15 days after the treatment, suggesting that high levels of PAA treatment could lengthen the product shelf-life. In a more recent study, electrolyzed oxidizing (EO) water and lactic acid were tested for *Campylobacter* reduction from naturally infected carcasses. A significant reduction of 1.31 $\log_{10}$ CFU/carcasses was observed when carcasses were overwhelmed in EO water after scalding. Also, after the inside−outside bird washer processing step, carcasses were overwhelmed in a 1.5% lactic acid solution showed significant reductions by 1.6−1.2 $\log_{10}$ CFU/carcasses by qPCR and enumeration detection methods, respectively. Chemical spraying has also been tested but no significant results were obtained (Rasschaert et al., 2013).

Postchill immersion treatments have also been evaluated with chlorine, PAA, or lysozyme on experimentally contaminated carcasses (Nagel et al., 2013). PAA treatments (0.1% and 0.04%) were significantly the most effective with 1.9 and 2.0 $\log_{10}$ reduction of *C. jejuni* from carcass rinse samples, respectively, compared to the positive control. Other treatments led to a 1 $\log_{10}$ reduction of *C. jejuni*.

Automatic spraying is another alternative for chemical decontamination of carcasses. However, to be effective, most parameters of the system need to be controlled and effectiveness also depends on the sample characteristics such as the contamination level, the tissue nature, and temperature.

Despite the above experimentations, none of these chemical treatments is authorized for poultry carcass decontamination in the EU due to a lack of proof and the limited effects of studied chemicals (Eurosurveillance editorial team, 2010). Also, the vast majority of studies focused on

experimentally contaminated carcasses whereas treatments on naturally infected ones were not evaluated.

In another respect, the ability of phages to reduce *Campylobacter* contamination level has been evaluated on chicken skin. Skin pieces infected by 10^4 *C. jejuni* C222 and treated with 10^6 PFU/cm^2 of phages 12673 led to a significant reduction of the *C. jejuni* colonization compared to the control group (Goode et al., 2003). In the same way, 10^4–10^6 *C. jejuni* NCTC 12662 inoculated skins, treated with 10^7 PFU of phage φ2 showed a decrease in bacterial load by 1.1 to 1.3 $\log_{10}$ compared to the untreated group and when skin samples were stored at +4°C. With −20°C storage, the decrease is higher and reached approximately 2.4 $\log_{10}$ (Atterbury et al., 2003).

A significant improvement of carcass contamination could be achieved with the implementation at the slaughterhouses of simple corrective measures, combined with possible hygiene criteria which, once applied, would discard the heavily contaminated batches (more than 10^4 CFU/g) from the broiler food chain, while the others would require some corrective measures.

7.4.2 Definition of Food Safety Criteria for *Campylobacter* in Poultry Meat

At a governmental level, food safety control for public health protection covers the range of different food chains related to a certain food products or product groups, including all relevant producers, manufacturing sites, and food service establishments within the country, as well as those importing into the country. With the increasing international trade in food and the fact that manufacturing sites in one country may provide raw materials to other manufacturers or finished goods (products) for large numbers of consumers living in importing countries, it is critically important that there be a harmonization of food safety control procedures. Safe food is produced by adhering to good hygienic practices (GHP), good manufacturing practices (GMP), good agricultural practices (GAP), and implementation of food safety risk-management systems such as hazard analysis critical control points (HACCP). However, the level of safety that these food safety systems are expected to deliver has seldom been defined in quantitative terms (van Schothorst et al., 2009). Therefore, in 2002, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) held a joint consultation meeting to explore the principles and to establish guidelines for incorporating microbiological risk assessment in the development of food safety standards, guidelines, and related texts. In this consultation, concepts such as ALOP and food safety criteria were discussed in detail. In 2003, the CAC adopted the Guidelines for the Judgment of Equivalence of Sanitary Measures Associated with Food Inspection and Certification Systems (CAC, 2003). Later, in 2004 it defined the so-called FSO and PO (CAC, 2004).

The Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs requires that food business operators ensure that foodstuffs comply with the relevant microbiological criteria, which are not based on risk analysis. In fact, in the Regulation 2073/2005, a microbiological criterion is a criterion defining the acceptability of a product, a batch of foodstuffs or a process, based on the absence, presence, or number of microorganisms, and/or on the quantity of their toxins/metabolites, per unit(s) of mass, volume, area, or batch. The same Regulation reports a food safety criterion as a parameter defining the acceptability of a product or a batch of foodstuff applicable to products placed on the market. Moreover, it describes a process hygiene criterion as a criterion indicating the acceptable functioning of the production process. It sets an indicative contamination value above which corrective actions are required in order to maintain the hygiene of the process in compliance with food law. It allows us to show that food safety management systems, such HACCP and the prerequisite systems GMP and GHP are working properly (van Schothorst et al., 2009).

The microbiological criteria reported in the European Regulation No 2073/2005 and the risk-based metrics, formulated by the CAC, are two different concepts (Manfreda and De Cesare, 2014). In the context of food safety, an ALOP is a risk-based metric and corresponds to a governmental expression of its national public health goal for foodborne risks, presenting a level above which the risk is unacceptable (Todd, 2003). Typically, an ALOP would be articulated as a value related to the disease burden associated with a particular hazard−food combination and its consumption in a country. Gkogka et al. (2013) showed two different risk-assessment approaches to derive a potential ALOP for *Salmonella* in chicken meat in the Netherlands. An ALOP, expressed for instance as a number of illnesses in a population per annum, or risk per serving, is not a measure that is meaningful for food safety management in practice. The food safety professionals responsible for controlling the specific hazards possibly associated with the food ingredients they use or the food products they market need more specific guidance from food safety control authorities (Gorris, 2005). To that end, and within the risk-analysis framework, it was proposed that, when deemed appropriate, competent authorities can formulate a so-called FSO. An FSO has been defined by the CAC (CAC, 2004) as the level of a hazard (in terms of concentration and/or frequency) that can be tolerated in the final product when it is consumed. Setting an FSO at the moment of consumption is supported by the ICMSF, as that is the moment when no change in the hazard level can occur anymore and essentially the consumption event is required to have a possible impact on public health (ICMSF, 2002). Because, conceptually, an FSO should be derived from the ALOP, there is a need for additional milestones that ensure the appropriate frequency and/or concentration of a hazard at specific steps along the food chain. This need has been

addressed with the POs that should be based on the FSO. The codex defines a PO as "the maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before consumption that provides or contributes to an FSO or ALOP, as applicable." A PO may be the same as the FSO if the frequency/concentration of the hazard stays at the same level between the point of the PO and FSO. Otherwise, Codex guides the PO to be more or less stringent than the FSO, according to the likeliness of the hazard to increase or decrease between the PO and FSO (CAC, 2005). In contrast to the ALOP and FSO, a PO may be established not only by the government but also by the individual food businesses. Thus, insight into the occurrence and behavior of the specific hazard throughout the chain is needed in order to derive POs at various points along the food chain to obtain the intended FSO (at consumption) (Van der Fels-Klerx et al., 2008).

Each safety criterion must be defined for a specific food/hazard combination. To calculate a food safety criterion, a representative number of food lots must be analyzed for prevalence or enumeration of the hazard of interest. The whole approach for PO calculation for *Campylobacter* in broiler carcasses tested after chilling has been published (De Cesare et al., 2015). The study included data from 10,162 carcasses belonging to 3400 batches sampled in 25 different countries during an EFSA survey performed in 2008 (EFSA, 2010a). The POs were set for carcasses collected immediately after chilling. A deterministic approach was selected to derive a potential FSO (ICMSF, 2002) to be used as a benchmark to elucidate POs for *Campylobacter* in carcasses corresponding to -1.2 $\log_{10}$ CFU/g, corresponding to c. 6 CFU/100 g. The *Campylobacter* concentrations after chilling resulting in a final concentration equal or below this proposed FSO are suggested as possible POs. For derivation of POs, *Campylobacter* is assumed not to be growing after chilling but eventually decreases between 1 and 4 $\log_{10}$ CFU/g up to consumption. The setting of a PO at the end of the slaughter line, after carcass chilling, can take into account the effectiveness of any postharvest intervention during processing. Moreover, this stage can provide quantitative indicators of bacterial load on the carcass, which would give the most valuable information to incorporate into risk assessment models and to assess the effectiveness of interventions.

To derive POs for *Campylobacter*, representative factors influencing *Campylobacter* counts and prevalence on carcasses (EFSA, 2010b; Habib et al., 2012) were taken into account. Such factors included slaughterhouse capacity, application or not of the thinning method, time between sampling and analysis. To describe the uncertainty of the number of positive carcasses within the same country, a Beta distribution was used for prevalence of *Campylobacter*-contaminated broiler carcasses (Vose, 2008). Moreover, a normal distribution of log-transformed enumeration data of *Campylobacter* in each EU country was calculated using maximum likelihood estimation (MLE) for left-censored data (i.e., those data that are positive with

a concentration below the limit of quantification of 10 CFU/g) (Busschaert et al., 2010).

The strategy followed to find the concentration levels in the food chain allowing meeting of the calculated FSO consists of applying the standardized equation proposed by the ICMSF (2002):

$$H_0 + \Sigma I + \Sigma R \leq FSO \tag{7.1}$$

where H_0 is the initial contamination (it was assumed that it corresponded to the estimated log-normal distributions for concentration), ΣI is the sum of increments and ΣR is the sum of reductions. After chilling of carcasses, no growth of *Campylobacter* was assumed before consumption, but different $\log_{10}$ reduction levels (ΣR). During the simulation, each reduction level was applied individually and the number of positives after reduction was calculated by considering three different sampling sizes ranging between 10 and 100 g. According to the possible reduction scenarios and different sample sizes, the possible POs ranged between -0.152 $\log_{10}$ CFU/g (c. 7 CFU/10 g) and 5.671 $\log_{10}$ CFU/g (c. 4.7×10^5 CFU/g) (De Cesare et al., 2015) (Table 7.2). No significant differences were observed according to the number of slaughtered broilers per year ($>10^7$ or $<10^7$) in the establishment of POs. However, a higher impact of the sampling size (w) and the reduction applied (R) was observed, since PO values range from -0.152 to 3.506 $\log_{10}$ CFU/g. A similar trend was obtained for the application of the thinning method, where a reduction between 3 and 4 $\log_{10}$ CFU/g implies an increase of the PO values in more than 0.5 $\log_{10}$ units when no thinning is applied. The impact of the time between sampling and analysis (*Time*) was not significant, as slight variations in the PO values were obtained (De Waal and Robert, 2005).

7.5 FUTURE TRENDS

1. In order to face the *Campylobacter* challenge, countries worldwide, in conjunction with industries and policy makers, should implement specific *Campylobacter* targets and/or *Campylobacter* criteria. Besides traditional bacterial pathogens, in recent years special focus has addressed *Arcobacter* and *H. pullorum* as emerging biological hazards in poultry meat.
2. Metagenomic analyses in combination with other approaches will allow the identification of the real players among GIT microbiota that help in host metabolism and in maintaining its health. Moreover, metagenomic investigations can provide a wide view toward the control of diseases in poultry as well as research related to enteric foodborne pathogens.
3. Feed additives (i.e., essential oils, organic acids, probiotics) can be used as alternatives to antibiotics for disease control in poultry but a scientific validation of their efficacy is required.

TABLE 7.2 Performance Objectives (log_{10} CFU/g) Estimated for the Different Datasets Considered at the Three Reduction Levels (*R*) and Sampling Sizes Studied

Risk Factor	Reductions (log10 CFU/g)								
	1-2R			2-3R			3-4R		
	10 g	25 g	100 g	10 g	25 g	100 g	10 g	25 g	100 g
Sl ($>10^7$ broilers/year)	−0.09	−0.11	−0.14	1.34	1.11	0.94	3.51	2.94	2.36
Sl ($<10^7$ broilers/year)	−0.130	−0.14	−0.15	1.17	0.99	0.88	3.33	2.73	2.17
Thin (yes)	−0.14	−0.14	−0.14	0.92	0.86	0.84	2.61	2.13	1.94
Thin (no)	−0.02	−0.10	−0.14	1.44	1.21	0.99	3.37	2.95	2.46
Time (>36h)	−0.04	−0.10	−0.14	1.55	1.25	0.99	3.32	2.95	2.51
Time (<36h)	0.05	−0.05	−0.11	1.50	1.28	1.06	3.86	3.25	2.54
Conc (high)	−0.15	−0.15	−0.15	0.91	0.86	0.85	2.46	2.15	1.92
Conc (low)	0.91	0.53	0.14	3.33	2.76	1.93	5.67	5.45	4.44

Sl, slaughterhouse capacity; *Thin,* thinning method; *Time,* time between sampling and analysis; *Conc,* contamination level of the broiler carcasses.

4. Genomic selection toward the improvement of antibody response to viral and bacterial disease can be used in order to produce food animals without antibiotics.
5. New vaccination strategies based on the identification, by sequencing analysis, of new antigens can protect animals from *Campylobacter* colonization. However, this new promising tool requires further research.
6. In order to increase the food safety for consumers the new risk-based metrics such as the ALOP, FSO, and PO seem to be essential. These targets can be estimated by using risk-assessment models, therefore, the research to implement such models should be a priority for scientists involved in food safety. The implementation of risk-based metrics requires an integrated approach in risk assessment, in management, and above all in risk communication. This means new challenges in the ways in which scientists, politicians, policymakers, and food operators interact.

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Part II

New Techniques for Measuring/Predicting/ Producing Meat Quality, and How They Help Us Minimize Variability in Eating Quality and/or Maximize Value

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Chapter 8

Genetics and Genomics for Improving Poultry Meat Quality

Elisabeth Le Bihan-Duval and Cécile Berri
URA, INRA, Nouzilly, France

8.1 INTRODUCTION

The quality of the product has been taken into account for a long time in poultry meat production and has strongly contributed to the development of diversified systems of production such as the standard, certified, free-range, or organic productions. Not only the conditions of production but also the quality of the product differ between these different systems which use a wide range of breeds slaughtered at various stages of the birds' maturity. For example, French Label Rouge chickens have to be reared up to a minimum of 81 days of age which results in a higher sensorial quality of the meat (Touraille et al., 1981a, b). In contrast, in standard production, selection for higher growth rate has led to birds which are slaughtered at a less mature stage, with meat which is usually more tender and juicy but shows a less intense flavor. As highlighted by Castellini et al. (2008), organic production is not only associated with good health status, animal welfare, and environmental concerns, but also with a good taste of the meat. In case meat quality is not fulfilled, it can raise barriers to organic production development. This confirms again that not only intangible attributes but also tangible attributes of the product quality have to be ensured, whatever the system of production.

Expectations in terms of meat quality attributes have evolved in the past decades in relation to the market demands. As previously occurred in North America, the European processing industry has gradually increased the availability of a large variety of processed ready meals, even if their distribution is still higher in the countries of northern Europe (United Kingdom, Germany) than in the countries of southern Europe (Spain, Italy, France) (Petracci et al., 2009). At the same time, as highlighted by the last authors, the European poultry market exhibits some peculiarities in respect to the North American market, such as the consumers' preference toward meat products with a recognizable meat structure and a European policy encouraging

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the limitation of the use of food additives and favoring the preparation of minimally processed foods. Thus, the poultry industry has to combine a higher degree of meat processing with the consumer's demand of a more "natural" product. This has underscored the necessity for a higher standard of quality to improve the sensory and functional properties of the meat (Fletcher, 2002).

Meat quality results from complex interactions between the genotype of the animal and its environment. At the genetic level, the first level of approach to improve meat quality is to take advantage of the diversity of breeds or strains in order to exploit valuable animal characteristics. The second level of approach consists of taking advantage of the within-breed genetic variability in order to improve meat quality through genetic selection. Efficacy of selection will largely depend on the access to quick and, if possible, nondestructive measurements of meat quality to avoid a heavy and costly sib-selection. This highlights the interest in identifying biological or genetic markers of poultry meat quality that will be helpful for genetic or genomic selection programs aimed at limiting the incidence of meat-quality defects or increasing favorable meat-quality attributes in the selected lines. This chapter will deal with several research programs by highlighting a strong genetic control of meat quality and new technologies in order to better understand the biological and genetic mechanisms unraveling the variability of meat quality.

8.2 EVIDENCE OF A GENETIC VARIABILITY OF MEAT QUALITY IN CHICKEN

In response to the increasing demand for poultry meat, quite significant progress has been accomplished in order to obtain more efficient birds which grow faster with a limited amount of feed. As reported by Kuttappan et al. (2016), while in 1925 the average market live weight was 1.1 kg for a 112-day broiler, in 2015 a market weight of 2.8 kg was achieved at 48 days. The shift of consumers purchasing from the whole bird to cut up parts has also led to more pressure on the proportion of breast meat, which over the last 10 years has increased by almost 5 points, and today represents more than one-fifth of bird weight (Petracci et al., 2015). Implications of intensive selection for higher growth and muscle development on muscle characteristics and meat quality is an important issue which has first been addressed by the comparison of different genetic lines, such as divergent lines issued from the same base population but selected in opposite directions.

8.2.1 Selection for Growth and Muscle Development: Impact on Muscle Fiber Characteristics

As underlined by Berri (2000), muscle function and meat quality are strongly determined by the histological and biochemical characteristics of muscle

fibers. Thus, structural, functional, and metabolic differences exist between red and white fibers (Dransfield and Sosnicki, 1999): (1) red fibers are narrow in diameter, myoglobin rich, and adapted to aerobic metabolism for fatigue-resistant activity; (2) white fibers are larger in diameter, rich in glycogen but poor in myoglobin and lipid, and adapted to anaerobic metabolism used for brief bursts of activity. With such differences, we expect a tenuous relationship between muscle fiber characteristics and meat quality. Quantitative and qualitative properties of two metabolic types of muscles, the *Pectoralis major*, a pure fast-twitch muscle, and the *anterior Latissimus dorsi* (ALD), a completely slow tonic muscle, were examined in an extreme model of chicken lines divergently selected for "high" or "low" body weight (1882 g vs 675 g at 77 days) (Rémignon et al., 1995). Differences in muscle mass obtained by this selection appeared to be due to changes in both fiber number (with an estimated increase of 20% in ALD) and fiber diameter, which was twice as high in the fast-growing (FG) birds than in the slow-growing (SG) birds. As the total number of fibers remained unchanged after hatching, this suggested that selection had influenced the *in ovo* development. Post-hatch development was also modified with a greater hypertrophy of muscle fibers in birds which grew faster. Whatever the muscle, selection for growth did not modify the myosin isoform profiles nor the activity of the enzymes involved in the glycolytic (i.e., lactate dehydrogenase and citrate synthase) or the oxidative (i.e., β-hydroxyacyl coenzyme A dehydrogenase) metabolic pathways. A complementary study was conducted to define variations of muscle fibers between two chicken lines significantly differing for breast weight (205.0 g vs 150.4 g at day 42) but close for body weight (1484 g vs 1419 g) (Guernec et al., 2003). The study of this model aimed at estimating the specific effect on muscle fibers of the selection for higher breast yield, a major criterion for selection in heavy meat-type strains. Chickens selected for higher breast yield showed larger fibers than their controls in breast as well as in *Sartorius* muscle, a mixed oxidative and glycolytic leg muscle. The amplitude of the difference increased with age, fibers of the selected birds being 24% larger at 6 weeks of age. In contrast, estimation of the fiber number index in breast did not differ between both genotypes. Moreover, the activity of the enzymes used as markers of glycolytic and oxidative pathways was not significantly modified (Berri et al., 2001).

These phenotypic results led to the consensual conclusion that the increased breast muscle mass obtained by selection has mainly been achieved by muscle fiber hypertrophy. This was confirmed at the genetic level too, since fiber size evaluated by its cross-sectional area was shown to be highly heritable ($h^2 = 0.41$) and strongly positively correlated with weight gain ($r_g = 0.69$), breast muscle weight ($r_g = 0.76$) and breast muscle yield ($r_g = 0.48$) in an FG standard chicken line (Le Bihan-Duval et al., 2008). If the comparison of lines did not support obvious changes in the metabolic profile of muscle fibers in response to selection, physicochemical

characteristics of the muscle have evolved with implications on postmortem metabolism and meat quality.

8.2.2 Between Line Variability of Muscle Postmortem Metabolism and Subsequent Meat Quality

In poultry as in pigs, variations in the onset of *rigor mortis* may significantly affect the technological quality of the meat, which refers to several properties including water-holding capacity (WHC), intensity, and homogeneity of color, firmness, shelf-life, and processing yields. Meat quality is closely related to the postmortem decrease in muscle pH. Rapid postmortem decline (evidenced by low pH value measured 15 min postslaughter in poultry, i.e., pH15) results in PSE (pale, soft, exudative) meat with a pale aspect and reduced WHC (Pietrzak et al., 1997; Owens et al., 2000). Variations in the extent of decrease in pH are also responsible for variations in meat quality. Low ultimate pH (measured 24 h postslaughter; pHu) results in "acid meat" which is often qualified as PSE-like since it presents similar defects (Barbut, 1997), while high pHu leads to dark, firm, dry (DFD) meat with dark color and poor storage quality (Allen et al., 1997). Comparisons of genetic lines have been conducted in order to evaluate the impact of selection for growth and/or body composition on postmortem metabolism and meat quality and to decipher the underlying physiological mechanisms.

This was the approach retained by Berri et al. (2001) who compared the breast meat metabolism and quality of four chicken lines: one experimental and one commercial line selected for increased body weight and/or breast meat yield, and their respective unselected "control" lines. Birds from the experimental selected line exhibited similar body weight but higher breast meat yield (+21%) and lower abdominal fat percentage (−25%) than birds from the control line (Table 8.1). Body weight as well as breast meat yield were much higher (+127% and +61%, respectively) in birds from the commercial selected line than in their control birds. Despite the fact that they corresponded to unrelated populations, quite similar trends were observed in the experimental and in the commercial genotypes. Significant variation in postmortem muscle pH fall was observed: by comparison with their controls, the two selected lines showed a lower rate and extent of pH decline during the postmortem period, which was consistent with the lower glycolytic potential they also exhibited. Despite higher values of pH at 15 min and 24 h postmortem, the breast meat of the selected birds was lighter and less red, most probably because of its lower heme pigment content. This change in the color did not reveal the presence of the PSE defect, since the meat of the selected birds did not exhibit excessive drip loss. In conclusion, this study evidenced changes in breast muscle metabolism in response to selection on growth and breast muscle yield, but did not support a higher incidence of metabolic defects, such as the PSE or acid meat, in the selected lines.

TABLE 8.1 Performance Traits and Breast Meat Quality Traits From the Experimental Selected Line (ES), its Corresponding Control Line (EC), the Commercial Selected Line (CS), and its Corresponding Control Line (CC)

Traits	EC	ES	CC	CS
Body weight (g)	$2237^{b} \pm 180$	$2223^{b} \pm 144$	$1306^{c} \pm 78$	$2966^{a} \pm 114$
Breast yield (%)	$12.5^{c} \pm 1.7$	$15.1^{b} \pm 1.9$	$11.5^{d} \pm 1.1$	$18.5^{a} \pm 1.2$
Abdominal fat yield (%)	$2.45^{a} \pm 0.76$	$1.84^{c} \pm 0.55$	$1.77^{c} \pm 0.50$	$2.09^{b} \pm 0.48$
pH15	$6.31^{c} \pm 0.13$	$6.42^{b} \pm 0.12$	$6.12^{d} \pm 0.14$	$6.55^{a} \pm 0.12$
pHu	$5.84^{c} \pm 0.14$	$5.90^{b} \pm 0.14$	$5.75^{d} \pm 0.12$	$6.03^{a} \pm 0.14$
L^*	$48.4^{b} \pm 1.6$	$49.8^{a} \pm 2.5$	$48.1^{b} \pm 2.3$	$49.7^{a} \pm 2.0$
a^*	$0.64^{a} \pm 0.86$	$0.20^{b} \pm 0.69$	$0.27^{b} \pm 1.1$	$-0.96^{c} \pm 0.61$
b^*	$10.86^{a} \pm 1.24$	$10.39^{b} \pm 1.18$	$9.38^{c} \pm 1.49$	$7.50^{d} \pm 0.80$
Drip loss (%)	0.90 ± 0.28	0.82 ± 0.29	0.86 ± 0.27	0.89 ± 0.30

pH15, pH measured 15 min postmortem; *pHu*, ultimate pH measured 24 h postmortem; L^*, lightness measured 24 h postmortem; a^*, redness measured 24 h postmortem; b^*, yellowness measured 24 h postmortem; drip loss measured 3 days postmortem.
^{a,b,c,d}*Means with the same letter in the same row do not differ* ($P > .05$).
From Berri, C., Wacrenier, N., Millet, N., Le Bihan-Duval, E., 2001, Effect of selection for improved body composition on muscle and meat characteristics of broilers from experimental and commercial lines. Poult. Sci. 80, 833–838.

Consistent trends were observed in the study of Berri et al. (2005a) who investigated the variability of meat quality parameters in relation with muscle characteristics of SG, medium-growing (MG), and FG chickens slaughtered at market ages of, respectively, 12, 8, and 6 weeks. The pH15 and pHu values of breast and thigh muscles were the highest in the 6-week-FG birds and the lowest in the 12-week-SG birds while the 8-week-MG birds were intermediate. By comparison with FG birds, breast meat was more colored (lower lightness and higher redness) in SG birds but was also characterized by higher drip loss. During processing, the highest curing-cooking yields were obtained with the breast and leg meat from 6-week-FG birds and the lowest with that from the 12-week-SG birds. As a consequence of their reduced WHC, the processed meat from 12-week-SG chickens exhibited the lowest moisture and the cured-cooked breast meat showed the driest texture and the best slice cohesiveness. This study revealed significant variations of postmortem muscle metabolism and meat quality parameters between the three genetics types, with FG birds being more adapted to further processing than SG birds in terms of profitability but maybe not in terms of quality of

the processed product. As shown by the studies of Debut et al. (2003) and Berri et al. (2005b), not only the muscle metabolism but also the behavior of the birds at slaughter could contribute to such variations. Strong negative correlations between wing flapping duration on the shackle line and pH15 were evidenced, showing that struggling hastened the initial rate of pH drop. By comparison to FG birds, the higher struggle of SG birds could be a disadvantage since it modified the color of the meat (by increasing redness) and impaired its WHC.

8.2.3 Estimation of Genetic Parameters of Meat Quality Traits

The ability to include meat quality traits in poultry breeding schemes depends on their level of heritability as well as genetic correlations with other economic traits. As illustrated in Fig. 8.1 for heritability values of breast meat pHu, studies of commercial and experimental broiler lines show that genetics is one of the most important determinants of meat quality traits. A preliminary study was conducted in 1999 on the previously described experimental line selected for increased body weight and breast meat yield and against abdominal fat percentage (Le Bihan-Duval et al., 1999). A predominant role of genetics was suggested for the pHu of the *Pectoralis major* muscle whose heritability value (h^2) was estimated at 0.49, as well as for meat color parameters (i.e., lightness L^*, redness a^*, yellowness b^*) whose heritability values ranged between 0.64 and 0.81. This study was refined a few years later in order to improve the accuracy of genetic parameter estimates by using a larger data base and to include new quality traits (Le Bihan-Duval et al., 2001). This new analysis confirmed the role of genetics in the determinism of pHu and color measurements, and showed

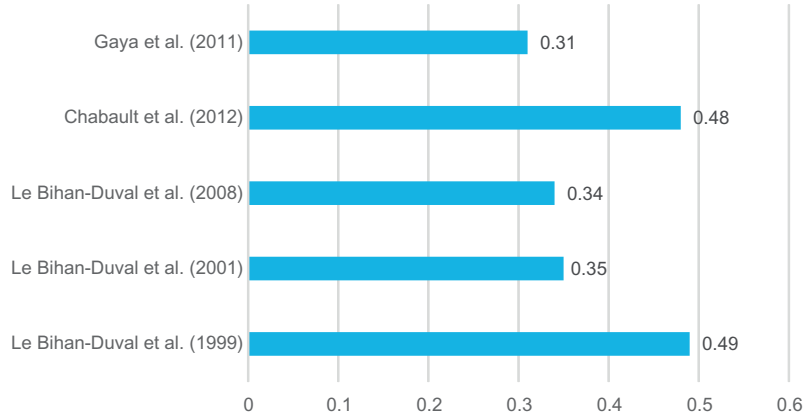


FIGURE 8.1 Heritability values of breast meat ultimate pH estimated in experimental or commercial chicken lines.

significant heritability values for drip loss ($h^2 = 0.39$) and pH at 15 min postslaughter ($h^2 = 0.49$). Genetic relationships between the different meat traits were also estimated showing that, in this genetic line, the rate and the extent of pH decline seemed to be controlled by different genes, as shown by the extremely low estimated genetic correlation (0.02) between pH15 min and pHu. In contrast, the pHu of the meat was genetically highly strongly related to its lightness (−0.91) and WHC (−0.83). These results suggested that selection for pHu could be exploited to prevent increased incidence of pale and exudative meat. Further studies in commercial populations confirmed a genetic basis of meat quality traits, even if their level of heritability varied according to the genetic lines and rearing conditions (Le Bihan-Duval et al., 2008; Chabault et al., 2012; Gaya et al., 2011). It is worthwhile noting that glycolytic potential of breast muscle measured at slaughter in an FG line appeared highly heritable ($h^2 = 0.43$) and highly genetically correlated (−0.97) with pHu, suggesting a common genetic determinism of both traits (Le Bihan-Duval et al., 2008). The study by Chabault et al. (2012) in an SG "Label Rouge" line confirmed the impact of behavior at slaughter on meat quality by showing that wing flapping duration on the shackle line was significantly heritable ($h^2 = 0.41$) and markedly genetically correlated with pH at 15 min postslaughter (−0.71) and redness (0.52) of meat.

8.2.4 Experimental Selection for Meat Quality

Based on these encouraging results, two independent experiments of selection were initiated in order to evaluate the feasibility of divergent selection for breast meat pHu (Alnahhas et al., 2014) or for breast meat lightness (Harford et al., 2014) measured 24 h postmortem. For both experiments, the criteria of selection exhibited high heritability values, which was estimated at, respectively, 0.47 and 0.51 in the thigh muscle color (HMC) and low muscle color (LMC) lines diverging for breast muscle lightness (L^*) (Harford et al., 2014), and at, respectively, 0.62 and 0.57 in the high pHu (pHu +) and low pHu (pHu −) lines diverging for breast meat pHu (Alnahhas et al., 2014). Consequently, significant genetic and phenotypic responses were obtained with quite consistent observations between the two experiments. After eight generations of divergent selection for L^*, this criterion was increased by 7 in the HMC line by comparison to the LMC line, while pHu was decreased by 0.4. Conversely, after eight generations of selection for pHu, this criterion was decreased by 0.6 in the pHu − line by comparison to the pHu + line, while meat lightness was increased by 7 which resulted in a paler meat (Fig. 8.2). As expected, both criteria (pHu and L^*) were negatively correlated, as illustrated by the genetic correlation of −0.47 estimated after five generations of selection in the pHu + and pHu − lines treated as a whole population (Alnahhas et al., 2014). Nevertheless, it is worthwhile noting that the genetic correlation was much

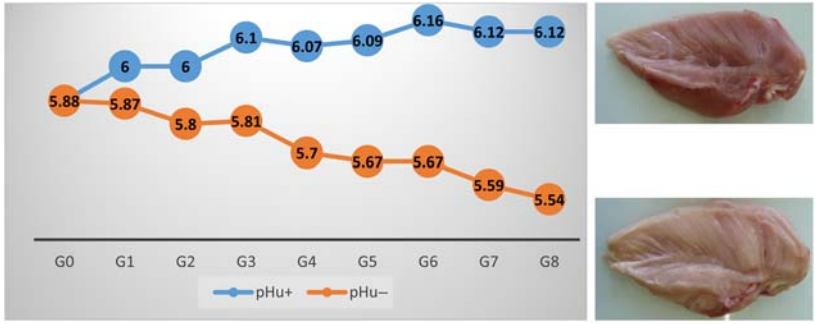


FIGURE 8.2 Breast meat ultimate pH can be efficiently modified by selection as shown by the phenotypic response observed over eight generations in the divergent pHu + and pHu − lines.

more pronounced in the pHu − line (−0.71) than in the pHu + line (−0.29), suggesting that factors other than the pHu could affect the variation of meat lightness in the latter line. In both experiments, an increase in meat yellowness (b^*) was observed for meat with lower pHu and higher lightness. Contrasted results were obtained for meat redness (a^*), since the increase in meat lightness was associated with a decrease in meat redness in the HMC by comparison to the LMC line, while the opposite trend was observed when comparing the pHu − line with the pHu + line. This suggested that the regulation of meat redness could be under different control between the two models. The variation of the physicochemical characteristics of the meat induced by divergent selection on meat lightness or pH decline largely altered the WHC, tenderness or processing ability of the meat. Thus, after eight generations of selection, the HMC line exhibited a higher percentage of fillet drip loss than both the LMC line and the control lines (Harford et al., 2014). After five generations of selection, breast meat of the pHu + line was characterized by greater curing-cooking yield, lower drip and cooking-loss and lower Warner−Bratzler shear force after cooking (Alnahhas et al., 2014). Regarding the sensorial quality, breast fillets of the pHu + line were more tender and had a less pronounced acidic taste than those of the pHu − line (Alnahhas et al., 2015). As far as growth traits were concerned, it was shown that selection had no impact on abdominal fat or body weight, while increasing pHu had a positive impact on meat yield (Alnahhas et al., 2014).

The fine phenotyping of pHu + and pHu − lines allowed a better understanding of the physiological changes induced by divergent selection. A strong genetic correlation (0.54) was observed between breast pHu (the criterion of selection) and thigh pHu, indicating a global rather than specific change in muscle energy metabolism in response to the selection. *Pectoralis major* muscle of the pHu − line was characterized by a higher glycolytic potential (+17%) compared to the pHu + line. Although the proximate composition (percentages of protein, lipid, and dry matter) of the

breast fillet was not influenced by the selection, the level of fat oxidation (estimated through the TBA-RS index) was significantly higher in the pHu − line than in the pHu + . Histologically, selection on breast pHu did not change the cross-sectional area of muscle fibers in the *Pectoralis major*, but it significantly reduced the number of capillaries per muscle fiber in pHu + compared to pHu − line (Alnahhas et al., 2015). This may have contributed to the decrease in the pHu + line of meat redness which is related to myoglobin content. Interestingly, additional observations showed that the structural and metabolic characteristics of the pHu + line could be predisposing factors to the development of white striping (WS), an emerging noninfectious quality defect characterized by the occurrence of white striations parallel to muscle fibers on breast, thigh and tender muscles of broilers (Kuttappan et al., 2016). Histological observations reveal that increased degree of WS is associated with an increase in the occurrence of chronic myopathic lesions along with lipidosis and fibrosis (Kuttappan et al., 2013). Beyond the deleterious impact on the visual appearance and nutritional value of products, this myopathy affects several quality parameters including color and water-holding and binding capacity of breast meat (Petracci et al., 2013, 2014; Kuttappan et al., 2016). WS was shown to be highly heritable ($h^2 = 0.65$) in the pHu + and pHu − lines, and highly positively genetically correlated with breast-meat yield and body weight which appeared to be the major determinants of this defect (rg = 0.68 and 0.33, respectively) (Alnahhas et al., 2016). The intramuscular fat content of the *Pectoralis major* was also strongly correlated with the defect (rg = 0.64), which was consistent with the presence of lipidosis described in affected muscles. As shown in Fig. 8.3, a higher incidence of moderate and severe cases of WS was observed in the pHu + line by comparison to the pHu − line, which is in line with previous results that showed that white striped breast fillets were characterized by higher pHu than normal breast fillets (Petracci et al., 2013; Mudalal et al., 2015). Moderate and positive genetic correlations were observed with pHu of the breast (0.21) and the thigh (0.31), which confirmed again that the incidence of this defect was not independent of muscle energy metabolism.

As proven experimentally, a sib-selection can efficiently be conducted in order to monitor the level of meat quality evaluated through indicators such as meat lightness or pHu. As already discussed by Harford et al. (2014) for L^*, these parameters can be selected in a direction that moves the population away from the risk of developing acid (or PSE-like) or DFD-like issues. The evidence of a relationship between high meat pH and the occurrence of new emerging defects, such as WS and wooden breast (WB) (Abasht et al., 2016; Alnahhas et al., 2016) may also indicate pHu as an important criterion to help to reduce meat quality defects in poultry by selection. Stabilizing selection aimed at removing extreme families is another interesting concept, whose efficiency to improve meat quality uniformity remains to be further evaluated.

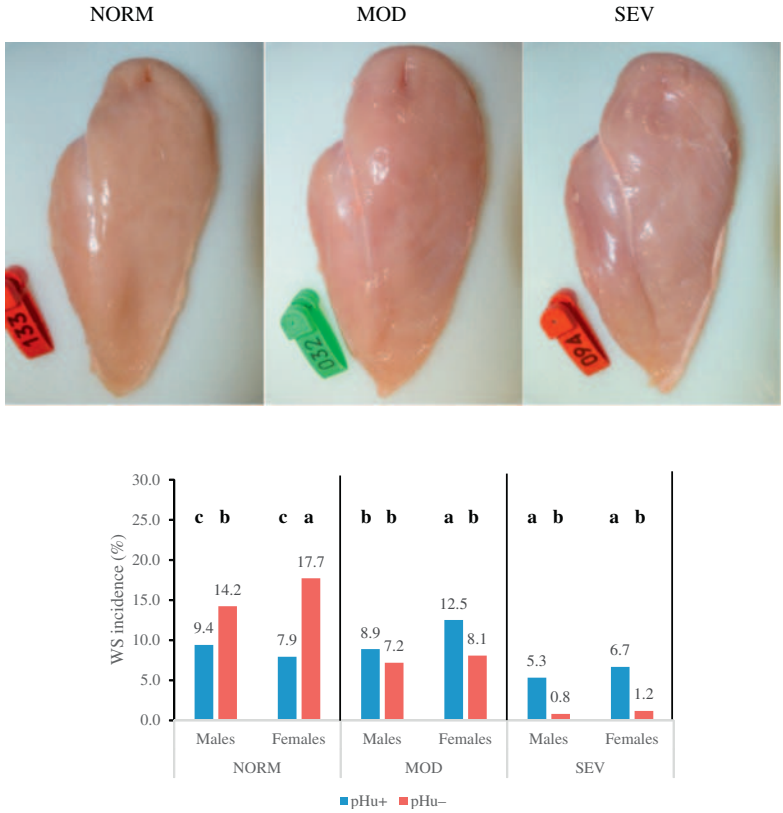


FIGURE 8.3 Scoring of normal breast fillets (NORM), breast fillets moderately affected (MOD) and severely affected (SEV) by white striping (WS) in the pHu + line selected for high value of ultimate pH and the pHu − line selected for low value of ultimate pH. The pHu − birds exhibit higher percentage of normal breast fillets while the pHu + birds exhibit a higher percentage of breast fillets severely affected by white striping. Observed frequencies per line and sex have been compared within each category of WS. Different letters indicate significant difference ($P < .05$) within each WS category. *From Alnahhas, N., Berri, C., Chabault, M., Chartrin, P., Boulay, M., Bourin, M.C., et al., 2016, Genetic parameters of white striping in relation to body weight, carcass composition, and meat quality traits in two broiler lines divergently selected for the ultimate pH of the pectoralis major muscle. BMC Genet. 17, 61.*

8.3 FUNCTIONAL AND POSITIONAL GENOMICS TO UNRAVEL MEAT QUALITY TRAITS DETERMINISM

While major genes such as the halothane sensitivity gene (Otsu et al., 1991) and the RN gene (Milan et al., 2000) have been shown to be responsible for PSE and acid meat defects in pigs, so far the genes and mutations underlying meat quality in poultry remain largely unknown. High-throughput technologies have been developed in chicken as in other animal species, which

makes possible to do the link between gene expression profiles or genetic polymorphisms revealed at the genome level with variations of meat quality phenotypes. These new approaches can help to unravel the molecular pathways underlying meat quality traits and to define new tools to monitor and improve them by breeding conditions and selection.

8.3.1 Transcriptomic Approach (Microarray, RNAseq)

Over the last 15 years, studies have been undertaken based on the use of high-throughput methodologies to gain a better understanding of the genetic basis (gene mutation or genetic marker) and the biological processes involved in the control of meat quality. These studies were mainly carried out in chickens, due to the greater availability of tools for this species (SNP and DNA chips, in particular) and to the growing importance of broiler production in the poultry industry. The first high-throughput approaches were devoted to the biological mechanisms involved in quality-related changes due to variations in postmortem pH and glycogen store of the muscle. More recently, studies have been carried out in connection with the appearance of new myodegenerative defects (WS, WB).

Researches on muscle transcripts were initially based on the use of DNA microarrays that are currently composed of 60,000 oligonucleotide probes and allow the simultaneous analysis of about 40,000 unique genes. The expression of the genes can also be quantified by sequencing techniques (RNA-seq), which makes it possible to obtain additional information on sequence polymorphisms. The transcriptomic analyses were mainly carried out on the *Pectoralis major* breast muscle which is the most concerned by the occurrence of meat quality defects. Most of the time, comparisons were made by considering groups of extreme animals in terms of meat quality either issued from experimental or commercial pure lines or crosses.

A first network of genes related to variations in breast meat pHu was obtained by comparing the muscle transcriptome of two experimental chicken lines divergently selected for abdominal fatness, that in addition to exhibiting a very different peripheral adiposity have different muscle glycogen contents (Fat > Lean) and specific meat quality characteristics (Sibut et al., 2008). The study highlighted the intervention of several important pathways for the control of glycogen in the muscle as the AMP-dependent pathway involving the AMP-activated protein kinase (AMPK) but also signaling pathways dependent on cyclic AMP or involved in controlling the availability of carbohydrates in muscle (Sibut et al., 2011).

To get out of the relationship between peripheral fattening and muscle glycogen storage capacities, the pHu − and pHu + lines specifically selected for low or high breast meat pHu have been created (see description above, Alnahhas et al., 2014, 2015). Recent nuclear magnetic resonance (NMR) analyses of both *Pectoralis major* muscle and serum showed very

specific metabolic signatures in both lines. The observed changes have allowed an integrated view of the preferred metabolic pathways for energy production in high and low pHu muscles (Beauclercq et al., 2016). Thus, if the pHu − line essentially uses metabolic pathways based on the use of carbohydrates, the pHu + line uses other mechanisms involving muscle proteolysis, amino acid catabolism, oxidation processes, including lipid peroxidation, which in turn stimulates the processes of response to oxidative stress in the muscle. In addition, analysis of the muscle transcriptome of pHu + and pHu − animals revealed nearly 1500 genes differentially expressed between lines. Functional analysis confirmed the intensive use of carbohydrate metabolism in the muscles of the pHu − line and the use of alternative catabolic pathways to produce energy in chicken muscle pHu +. Interestingly, this analysis also revealed that a large number of genes overexpressed in the high pHu muscles are involved in muscle remodeling and the response to oxidative stress suggesting that an energy deficit could compromise the development and integrity of muscle tissue. This was confirmed by joint histological gene expression analyses that revealed a more intense process of muscle fiber regeneration in the high pH muscle compared to the acid muscles (Beauclercq et al., 2017).

A differential analysis of the muscle transcriptome in relation with the WS occurrence was performed by comparing individuals based on their phenotype (presence or absence of defect) and their genotype (homozygous to a single-nucleotide polymorphism (SNP) marker identified by Quantitative Trait Loci (QTL) analysis as being involved in WS defect control) (Fig. 8.4). Functional analysis of the data revealed two major regulatory pathways potentially linked to the onset of the defect. The first one is the Wnt/Ca^{2+} pathway, which regulates cellular Ca^{2+} and early muscle development. The second one is a pathway involving the hepatocyte growth factor (HGF) that contributes to the activation of satellite cells (muscle stem cells) to form new fibers during muscle regeneration (Volonte et al., 2005). These observations may be related to the frequent presence of cellular regeneration foci in WS-affected muscles, which may involve molecular mechanisms similar to those activated during myogenesis. Moreover, among the biological functions revealed by the functional analysis, one participates in the muscular development and involves several networks of genes that contribute to the proliferation and the differentiation of the muscular cells. Finally, among the differential genes identified, some are involved in the appearance of various syndromes such as connective tissue hyperplasia or lipodystrophy (loss of subcutaneous adipose tissue), which have been shown to be associated with myopathic changes within the muscle (Rajab et al., 2010; Llamas-Velasco et al., 2012).

In an approach involving both transcriptomic (Mutryn et al., 2015) and metabolomic (Abasht et al., 2016) analyses, it was possible to describe precisely the main biological processes involved in the implementation of the

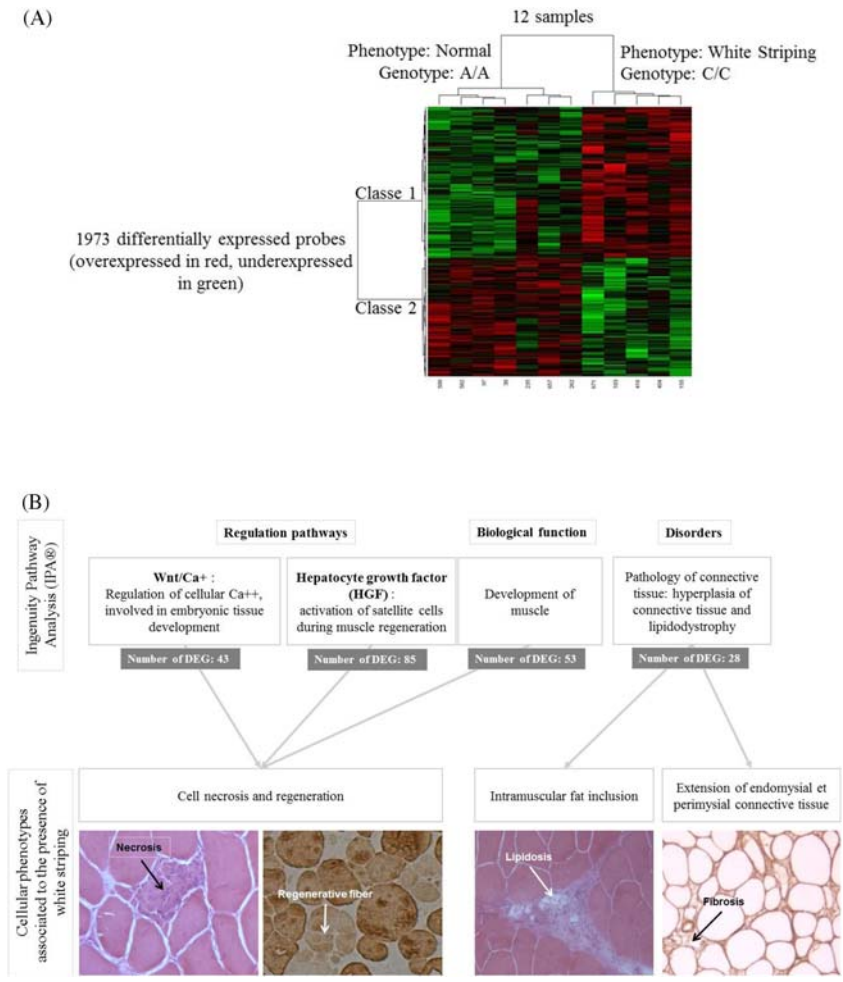


FIGURE 8.4 (A) Heat map from the hierarchical clustering of differentially expressed probes between control and white-striped chicken breast muscles. The scaled expression by row (gene) is shown as a heat map. It is reordered by a hierarchical clustering analysis (Pearson's distance and Ward's method) on both rows and columns. (B) Biological interpretation of expression data performed using Ingenuity Pathway Analysis 7.0 (IPA, Ingenuity Systems Inc., Redwood City, CA) in view of cellular changes observed in muscle affected by white striping.

WB defect. Thus, RNA-seq analysis of chicken fillets free from or affected by WB showed that the appearance of the defect was related to several biological processes. These include abnormal accumulation of intracellular calcium, localized hypoxia phenomena, and increased oxidative stress and accumulation of ROS (oxygen-reactive species). The appearance of the defect also involves changes in typology of fibers from fast to slow types,

increased cell repair and regeneration in skeletal muscle, negative regulation of genes linked to the coagulation system, and, on the contrary, overexpression of genes encoding different forms of muscle collagens. Coherently, targeted analysis by real-time PCR also revealed the overexpression of several genes involved in the organization of collagen and in the process of repairing lesions of muscle fibers involving satellite cells (Velleman and Clark, 2015). In agreement with all these results, a recent study described the transcriptomic changes related to the joint appearance of WB and WS. It confirmed that the differential genes expressed in the affected muscles are involved in the processes of inflammation, muscular degeneration and regeneration, oxidative stress, altered ionic homeostasis and glucose metabolism, lipidosis, fibrosis, and the synthesis of proteoglycans (Zambonelli et al., 2016).

Interestingly, observations made at the transcription (and metabolic) levels reveal common pathways involved in the physiological regulations on meat defects related to muscle energy deficit or growth potential. This implies an increase in protein degradation and the catabolism of amino acids that may induce processes of regeneration of the muscle fibers, with consequences in terms of contractile and metabolic typing. It also involved a decrease in the muscle vascularity that could be at the origin of their poor oxygenation, increased use of muscle carbohydrate, and an increase in oxidative stress and ROS production leading to a reduction in muscle redox homeostasis of muscle tissue.

8.3.2 QTL and eQTL Detection

During the last decade, research works have been developed in order to look for QTL controlling meat quality traits. The implications are a better understanding of the genetic architecture of meat quality and the development of molecular tools for selection as an alternative or improvement to the classical quantitative selection based on the sib-test. The Animal QTL database has been designed in order to house all publicly available QTL and SNP/gene association data on livestock animal species (Hu et al., 2013). It currently includes 6633 chicken QTL out of which only 105 concern meat quality traits. Studied traits are in relation to muscle fibers characteristics (including fiber density, number, diameter, cross-sectional area), muscle composition (including protein, dry-matter, and intramuscular fat contents) and technological characteristics of the meat (such as pH, color, WHC). Methodologies of QTL detection have evolved with genotyping technologies, going from within-family linkage analyses, which were adapted in case of microsatellite markers of low or medium density (Van Kaam et al., 1999; Rabie et al., 2005; Wright et al., 2006; Nadaf et al., 2007; Yoshida et al., 2013) to genome-wide association studies (GWAS) which became possible with the development of high-density SNP markers (Liu et al., 2013; Sun et al., 2013; Zhang et al., 2015). Several QTL were also detected by looking for

association between polymorphisms of functional candidate genes and quality traits (Nie et al., 2009; Zeng et al., 2011; Bai et al., 2012; Han et al., 2012; Wei et al., 2012; Chen et al., 2013a,b; Zhang et al., 2013; Huang et al., 2015; Liang et al., 2015). As underlined by Sun et al. (2013), both linkage analysis and candidate gene analysis have limitations since the identified QTL regions are generally large and require subsequent fine mapping to identify closely linked markers or causative variants. Moreover, candidate genes, based on putative physiological roles, may exclude the identification of novel genes or pathways that influence the target traits. In contrast, GWAS can aid in more precisely identifying candidate genes underlying the trait of interest and markers, which could be useful for marker-assisted selection or genomic selection.

Identification of causative genes and mutations is still a stimulating objective in view of its input for a better understanding of the biological determinism of quantitative traits and for progress in selection methods offered by gene-assisted selection or genomic selection including causative variants. However, while several hundreds of QTL have been reported in major livestock species, the identification of the genes and polymorphisms responsible for the variation of the phenotypes is still rare. Combining expressional and positional data can help in unraveling the causative genes that are affected by changes in DNA. This approach was applied with success to identify a causal gene underlying the yellow color of breast meat in chicken (Le Bihan-Duval et al., 2011). This research was conducted on a unique resource population obtained by a second-generation (F2) inter-cross between two experimental lines of chickens divergently selected for body weight. As detailed by Nadaf et al. (2007), at 9 weeks of age body weight and abdominal fatness were much higher in the High Growth (HG) line by comparison to the Low Growth (LG) line (3- and 12-fold differences, respectively). Interestingly, significant differences in breast meat quality were also reported, including for meat color which was significantly paler (higher L^*) and less intense (lower redness a^* and yellowness b^*) in the HG chickens. A large variability in meat quality traits was observed in the F2 inter-cross: for example, the difference between the F2 extreme birds was nearly 1 unit for meat pHu and 8 units for meat yellowness. Several QTL for meat quality traits including breast meat pH, drip loss, and color were identified in this F2 inter-cross (Nadaf et al., 2007). However, the confidence intervals of the QTL remained large, which prevented any possibility of marker-assisted-selection. Several regions of interest were then refined, including a genome-wide significant QTL for breast meat yellowness (BCo-Y) and redness (BCo-R) detected on chromosome 11. Thanks to the development of additional markers, a huge level of significance for BCo-Y QTL was confirmed and its position refined (Fig. 8.5). All the F1 sires were found to be heterozygous for BCo-Y QTL, the allele transmitted by the LG line having a positive effect, consistent with the more intense color of the meat observed in this

line. Similar refinement was obtained for BCo-R QTL, although the level of significance of this QTL remained lower. The BCo-Y QTL and BCo-R QTL overlapped, which suggested a strong impact of this chromosomal region in the variability of the breast meat color in the F2 population. After this step of refinement, a bioinformatics analysis of the BCo-Y QTL region was conducted and *BCMO1* was identified as a good positional and functional candidate gene (Fig. 8.4). This gene encodes for the β-carotene 15, 15′-monooxygenase, a key enzyme in the conversion of the β-carotene (a yellow color pigment) into vitamin A. The next step of the research was aimed at looking at the relationship between *BCMO1* gene expression and the variation of yellow color explained by the QTL. *BCMO1* mRNA level in breast muscle was found to be higher in HG chickens compared to LG chickens across several ages, which suggested a higher level of conversion of the β-carotene in the former line. Then *BCMO1* mRNA levels were quantified within a full F2 family (i.e., on more than 130 birds) and this molecular phenotype used for an expression QTL (eQTL) analysis. A strong cis e-QTL was detected and, by considering the mRNA level as a covariate, it was confirmed that the variation of *BCMO1* gene expression

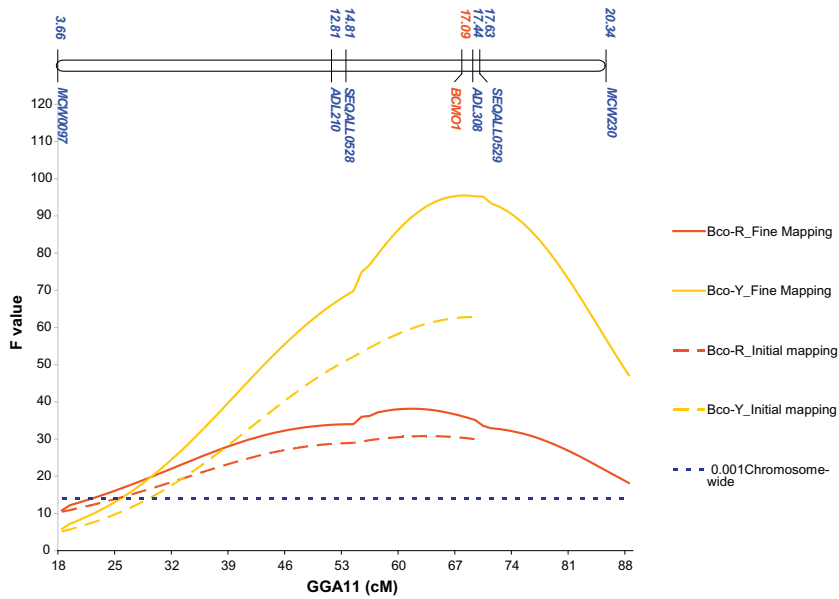


FIGURE 8.5 Fine mapping of color QTL controlling breast meat *yellowness* (light gray in print version) (BCo-Y) and *redness* (dark gray in print version) (BCo-R) on chromosome 11 in chicken. *From Le Bihan-Duval, E., Nadaf, J., Berri, C., Pitel, F., Graulet, B., et al., 2011. Detection of a Cis eQTL controlling BCMO1 gene expression leads to the identification of a QTG for chicken breast meat color. PLoS ONE 6(7), e14825, doi:10.1371/journal.pone.0014825.* BCMO1 *is identified as an excellent positional and functional candidate gene.*

was responsible for the variation of meat color due to BCo-Y QTL. To our knowledge, this is the first case (at least in one livestock species) in which a causative gene underlying a QTL has been identified thanks to an eQTL approach. The latter results also provided strong indications about the pathways of the causative mutations, which affected the level of transcription of the gene. Several putative mutations were identified within the gene by DNA sequencing. The functional effect of two SNPs in total linkage disequilibrium on the level of transcription of *BCMO1* gene was further confirmed by in vitro expression analyses. From all these results, it was concluded that this "double" mutation, localized in the promoter region of *BMCO1* gene, was a causative mutation responsible for variation of meat color in the chicken. A fast genotyping test of the double mutation through High-Resolution Melting (HRM) analysis was developed, which made the screening of the mutations in populations fast and cheap.

Another strategy, which also combined positional and expressional data, was performed in order to better characterize and to identify candidate genes for a pH15 QTL mapped on chromosome 1 within the same population (the F2 inter-cross between HG and LG lines). Little information is known on the genetic control of this trait in chicken and no obvious candidate gene was revealed by the bioinformatics analysis of this moderate-effect QTL whose confidence interval remained large. This simple strategy consisted in comparing the gene expression profiles of two groups of birds ($n = 12$ by group) showing homozygous divergent genotypes at the QTL (Nadaf et al., 2014). Microarray differential analysis of the two groups identified 10 significant probes that were exclusively seen in the QTL region (Fig. 8.6). Moreover,

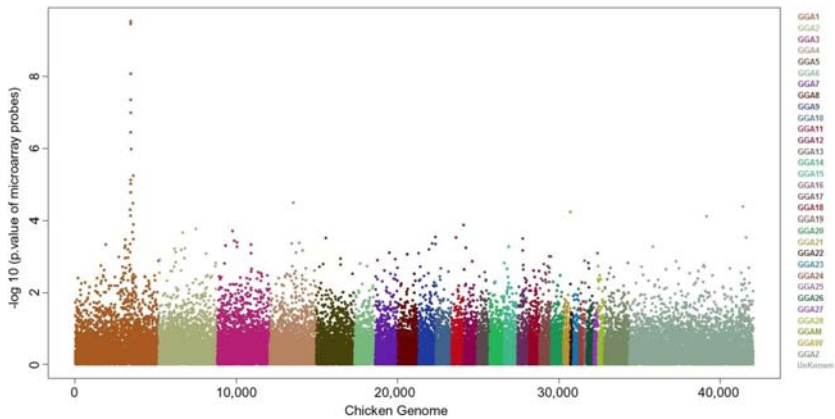


FIGURE 8.6 Manhattan plot of differential expression statistics ($-\log_{10}$ (*P*-value)) against the chicken genome. The signals are highly enriched at the Quantitative Trait Loci (QTL) position on chromosome 1. Different colors indicate different chromosomes. *From Nadaf, J., Berri, C., Dunn, I., Godet, E., Le Bihan-Duval, E., De Koning, D.J., 2014. An expression QTL of closely linked candidate genes affects pH of meat in chickens. Genetics 196 (3), 867–874.*

eight of these 10 probes were located within a small region (about 204 kb), showing a marked reduction of the QTL interval. Differentially probes within this small region targeted four known genes (*KLHL15*, *APOO*, *PRDX4*, *ACOT9*), whose function suggested that the QTL was controlling pH15 by governing oxidative stress. Resequencing of the region of interest for a few informative birds (10 birds) has allowed identifying candidate polymorphisms that could explain the genetic variability at this QTL (Li et al., 2015). A few of them (four) were nonsynonymous SNPs located within genes (including *PRDX4*). Others (54) were detected within gene-related regions (UTR regions, splicing sites, CpG islands, and promoter regions). In order to go further and differentiate among the current lists of candidate genes and mutations, other experiments should to be envisaged. For instance, the association between candidate SNPs and phenotypes should be tested on a broader panel of birds from different genetic lines.

8.4 CONCLUSION

The interest in research on the genetic control of meat quality has not diminished over the last decade. New challenges have emerged such as a better understanding of the origin of metabolic defects (related to the variation in postmortem pH drop) or structural defects (such as WS and WB). Qualitative differentiation remains an important issue for the competitiveness of strains and implies optimized selection on meat quality traits. Genomics offers new possibilities, from gene-assisted selection to genomic selection. Advances in genetics will also benefit from progress on phenotyping of meat quality and from the development of new indicators or biomarkers. Moreover, the emergence of new quality problems highlights the importance for the poultry sector of improving conditions of production and genetic lines in order to ensure more sustainable meat production.

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Chapter 9

Proteomics and Metabolomics in Relation to Meat Quality

Francesco Capozzi[1], Alessia Trimigno[1] and Pasquale Ferranti[2]
[1]*University of Bologna, Cesena, Italy,* [2]*University of Naples "Federico II", Portici, Italy*

9.1 INTRODUCTION TO PROTEOMICS AND METABOLOMICS

9.1.1 Definition of Proteomics and Metabolomics

During the last two decades, the so-called "omics" sciences emerged and have greatly developed in a broad number of research fields. Genomics and transcriptomics originated from the discoveries in genetics, focusing on the analysis of DNA and gene expression modulation, constituting also a basic approach in food research (Capozzi and Bordoni, 2013). Following this holistic approach, proteomics developed as the science capable of analyzing the whole set of proteins that is expressed in an organism or in its structures (organs, tissues, cells, enzymes) as well as of the related multiple isoforms and fragments thereof, also including the different posttranslational modifications (Paredi et al., 2012; Ferranti et al., 2015). It is clear how this science can be very useful in understanding biological processes and the influence of different genotypes and external factors on protein expression and how it is relevant for the definition of protein-rich food products (D'Alessandro et al., 2011). To further analyze the biological system from its genome to its biological functions, metabolomics arose as the research field that studies all the cellular molecules taking part in living cells reactions (metabolites) (Ferranti et al., 2015). Departing from the genome, modulated by external modifications and epigenetic alterations, it is necessary, in fact, to study the expression of the proteome, which constitutes, together with the metabolome, the whole living system. In effect, metabolomics is considered the thorough and concurrent determination of endogenous metabolites at the molecular level, and their global and dynamic changes over time, in complex multicellular systems as a consequence of biological stimuli or genetic manipulation or both (Nicholson and Lindon, 2008). These metabolites represent the mark of specific metabolic pathways and biochemical activities,

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giving insights into how a system's biochemistry responds to specific factors (diseases, drugs, diets) (Hu and Xu, 2013). The comprehensive knowledge of the three "omes" (genome, proteome, and metabolome) will result in great scientific progress, allowing us to learn how the whole biological system is regulated. By focusing all omics in the holistic definition of the food matter and its link to healthy nutrition, a new discipline has been coined in 2009 taking the name "Foodomics" (Capozzi and Bordoni, 2013). In the development of the advanced omics platforms, because of their potential to profile complex mixtures of biomolecules, mass spectrometry (MS) techniques have assumed an unquestionable role. The analytical capability of MS became highly descriptive, but still merely dependent on the resolution power of the coupled separation devices (high-performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE), supercritical fluid chromatography (SFC), polyacrylamide gel electrophoresis (PAGE)): synergistically together, spectrometry and chromatography are able to characterize, at the molecular level, the entire panel of the components of a complex system (Ferranti et al., 2015). Nuclear magnetic resonance (NMR) spectroscopy is the other elective platform for metabolomics and, although less sensitive than MS, provides further robust information about the whole system, as it does not necessarily require a separation step prior to analyses. For these reasons MS, together with NMR and other spectroscopic techniques, are the core essence of the omics technologies (Ferranti et al., 2015). Comprehensive analysis of omics data can give scientists the power to study not only individual biomarkers, but also the occurrence of patterns of proteins, lipids, and metabolites, useful in tracing a food from raw materials to the end products, also establishing structure/function links for the design of novel functional foods and to monitor food chain contamination by microbial and chemical agents (Ferranti et al., 2015).

9.1.2 Main Platforms Employed

9.1.2.1 Proteomics Techniques

"In the past ten years, scientifical and technological advancements have been made in order to find and perfect new methodologies capable of separating, purifying and characterizing different food samples" (Ferranti et al., 2015). In spite of this, food characterization still remains a challenge, due to the great variability of food matrices and related samples (i.e., source, type of food, storage parameters, processing, contaminants, sampling procedures) (Ferranti et al., 2015).

In biomedical samples, the most employed chemical and biochemical techniques for sample purification and analysis are GC, HPLC, CE, monodimensional (1D) or two-dimensional (2D) PAGE, and enzyme-linked immunosorbent assay (ELISA) (Ferranti et al., 2015).

Even though these methods can be extremely helpful for routine analyses, they can merely be descriptive. In fact, they can simply compare a reference

value or profile with the tested samples, analyzed employing the same conditions. In order to overcome these limitations, modern omic approaches were developed and are now available. Spectroscopic methods such as, most often, MS and NMR, usually combined with separation techniques and chemometric and bioinformatic tools, are the basis of omic approaches such as proteomics or metabolomics (Ferranti et al., 2015). In recent decades, MS has been employed more and more due to its high versatility, sensitivity and specificity, great informative level and capability of high-throughput screening (Hood et al., 2012; Ferranti et al., 2015; Porcari et al., 2016).

The output of MS technology is the molecular mass of an analyte, which is a key feature for any molecule. Moreover, with the information gained from the fragmentation of the molecules of interest, it is possible to identify the separated components of a mixture and even structurally characterize unknown compounds (Ferranti et al., 2015). In MS-based proteomics, for instance, it is possible to explore the whole range of proteins and peptides in a sample employing MS technologies coupled with a prior separation step (with high-resolution electrophoresis or chromatography).

There are two main ways to perform MS-based proteomic workflows. These strategies are called the bottom-up and the top-down approach (Angel et al., 2012; Ferranti et al., 2015) and differ for the type of MS instruments used and the separation required.

In the first one (bottom-up), generally a separation by two-dimensional gel electrophoresis (2-DE) is followed by the analysis of the peptides produced by in-gel proteolytic digestion (e.g., with trypsin) by matrix assisted laser desorption ionization-time of flight (MALDI-TOF) MS or liquid chromatography (LC)-MS/MS and subsequent database search (Peptide Mass Fingerprinting, PMF). The employment of 2-DE allows the resolution of complex proteomes, but it has the disadvantages of being very laborious and having a limited dynamic range (Ferranti et al., 2015).

Compared to the use of electrophoretic techniques, the coupling of LC with MS seems easier and more straightforward. In the past, MS has been generally coupled with GC to be used in both environmental and biological analyses. The determination of the most suitable form of LC to be coupled to MS or tandem MS depends largely on the application. For example, in the clinical screening of bile acids in urine, the best option could be to inject a crude extract. Most generally, in LC-MS applications, the ultimate stationary phase employed is a reverse phase (RP), while the mobile phase is usually acetonitrile or aqueous alcohol (Ferranti et al., 2015).

9.1.2.2 *Metabolomics Techniques*

Metabolomics studies the subset of metabolites (metabolome) with specific biochemistry and functions in the biological sample of interest. The metabolome, in fact, can be considered the entire metabolite cohort of an organism,

but also the metabolic profile of its specific parts, such as organs, tissues or cells. Blood and urine can be studied in their specific metabolome since they also are affected by both the genome and external perturbations. Due to the disparateness of characteristics and concentrations of those metabolites, suited analytical techniques are necessary for qualitative and quantitative metabolomics investigation depending on the studied metabolome (Hu and Xu, 2013). These technologies are required to be "high-throughput" and fast in the acquisition of a large number of measurements (Capozzi and Bordoni, 2013).

One of the main technologies employed is MS, which is usually chosen for its great sensitivity and high specificity, capable of the detection and quantification of even small-molecular and low-concentrated metabolites.

MS is usually coupled with techniques capable of separating metabolites from the sample, in order to be able to discriminate them. GC-MS is generally employed to analyze volatile components, while LC-MS is chosen for the metabolites that are not volatile at conditions compatible with their stability. Hydrophilic interaction chromatography (HILIC) is instead used for very polar or ionic molecules, while reverse-phase HPLC (RP-HPLC) is able to investigate both polar and nonpolar metabolites, such as lipids. New technologies in this field are represented by ambient desorption ionization (DESI) techniques since they allow the direct examination of the sample in open atmosphere with very little sample preparation, reducing therefore the time of analysis per sample (<1 min per sample instead of 15−60 min with GC- or LC-MS). Similar to DESI, there is direct analysis in real time ionization (DART), which represents one of the atmospheric pressure chemical ionization (APCI) methods and it is greatly employed for the soft ionization of both polar and nonpolar molecules (Cajka et al., 2013).

NMR spectroscopy is another high-throughput technique employed for metabolomics. NMR is in effect direct and fast and it is able to analyze a wide range of metabolites without much sample preparation (Hu and Xu, 2013). This technique is indeed able to provide the whole molecular profile of the sample and thus, the entire metabolome, and can be applied to a great range of both liquid and solid matrices without sample alteration nor the production of dangerous waste (Capozzi and Bordoni, 2013).

This technique began to be employed in organic chemistry in the late 1940s, in order to investigate molecular structure. In the 1980s, after the development of new instruments and the development of further knowledge, its application was expanded also to food science (Marcone et al., 2013) (Table 9.1)

9.1.3 Proteomics and Metabolomics in the Investigation of Meat Quality

Meat quality has been defined in the literature by four types of product characteristics: (1) nutritional value (i.e., content of fat, protein, carbohydrates,

TABLE 9.1 Advantages and Disadvantages of the Two Main Technological Platforms Employed in Metabolomics: Mass Spectrometry and Nuclear Magnetic Resonance (NMR)

	Advantages	Disadvantages
Mass spectrometry	• Higher sensitivity • Requires lower sample size • Machines cost less than NMR • More databases of spectral information	• High dependence on the experimental conditions (lab, technicians, etc.) • Quantitative analysis can only be comparative between samples • Need of separation techniques and sample preparation (usually lengthy)
Nuclear magnetic resonance	• Fast • Non-invasive • Low cost per experiment • Minimal sample preparation needed • Sample can be recovered • Information on the molecular structure (i.e., position of functional groups) can be gained • Quantitative	• Lower sensitivity • Expensive instrument • Greater sample size required

minerals, digestibility); (2) processing quality (pH, fatness, water-holding capacity (WHC), shear-force, length of sarcomeres); (3) safety (contaminants, residues, additives, microbiological status, etc.); and (4) sensorial quality (texture, color, flavor) (Becker, 2000) (Fig. 9.1)

These characteristics can be influenced by various intrinsic and extrinsic factors such as genetics (including gender, accounting generally for 30% of variation), breed, feeding, rearing conditions (space allowance, activity, climate, etc.), possible diseases, transport and preslaughtering conditions, type of slaughtering, postslaughtering handling (e.g., chilling and storage), meat processing (e.g., heat treatments), product formulation and additives (Olsson and Pickova, 2005). The per-capita consumption of poultry meat from 1990 to 2009 has increased by almost 80% and in future projections it is still expected to grow. The global trends in meat consumption show that income and price are factors that will lose their importance, while quality will become a greater influencing factor (Henchion et al., 2014). The quality of meat, in effect, has been recently increasingly recognized as a very important character and a great number of studies has been carried out to evaluate the

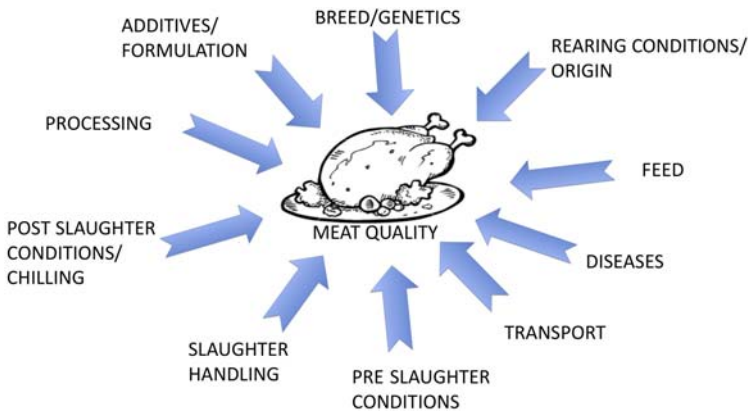


FIGURE 9.1 Factors influencing meat and meat-product quality.

link between the influencing factors and the main features of this quality such as texture, aroma, color, oxidation, and drip-loss.

9.1.3.1 Genetics and Breed

As stated, genetics and breeds have a great impact on the final characteristics of meat and meat products. Proteomic technologies in food science are capable of defining the entire and detailed biochemical composition of a food product and its modifications caused by specific factors (Mamone et al., 2013).

One of the tools emerging for the discovery of biomarkers and for food authentication is MALDI-TOF-MS, capable of performing a molecular profiling of a sample. Through this approach, it is thus possible to assess species and specific genetic lines. Traditional strategies, such as polymerase chain reaction (PCR), can therefore be complemented or substituted by MS-based technologies in studies regarding, for example, the identification of foodborne bacteria or the authentication of meat and fish (Mamone et al., 2013). Currently, there is a very poor presence in literature of applications of such approaches for studies focused on poultry meat. However, it is not speculative to affirm that the same protocols and methods so far employed for different meat (e.g., pork and beef) and seafood could be easily adapted to assess genetic origin and assist breeding schemes for the poultry sector.

Mazzeo et al., applied a newly developed MALDI-TOF-MS approach for the authentication of fish (Mazzeo et al., 2008). In this case, 25 different fish species were analyzed and their highly specific MS profiles were obtained. The biomarkers found were signals from protein with an approximate molecular weight of 11 kDa. The method thus developed can also be employed for the assessment of the authenticity of commercial products and to prevent fraudulent substitutions in species which are commonly subjected to this (Mamone et al., 2013).

Pineiro and colleagues (Piñeiro et al., 2003) employed proteomics to spot new protein markers for the authentication of seafood. Gel-based methods were used and a polypeptide was chosen to differentiate between hake species of high value and the ones considered less valuable. Other marker proteins were also suggested for the assessment of seafood freshness and shelf-life and to differentiate frozen fish from fresh ones (Piñeiro et al., 2003; Mamone et al., 2013).

Breed is an important factor in meat quality also because particular genetic lines are considered endowed with higher quality and therefore will be sold at higher prices to consumers. For this reason, fraudulent labeling occurs and tests to reduce this fraud have recently been developed and investigated. Many metabolomics studies have been applied to investigate meat in this sense. Straadt et al. employed high-resolution (HR)-NMR spectroscopy to investigate the potential of metabolomics in the assessment of the meat quality as revealed by the metabolic profile of new pig-breeds (uncommon and novel pig crossing between Iberian and Mangalitza vs the typical Duroc and Landrace pigs) (Straadt et al., 2011). The profile of meat, especially affecting the drip, revealed that the postmortem formation of lactate, measured through 1H-NMR techniques, is correlated to breed and this would also explain the differences in WHC. Ritota et al. employed 1H-NMR-magnetic angle spinning (MAS) in order to acquire the metabolic profiles of two beef muscles from four different breeds (Buffalo, Chianina, Holstein Friesian, and Maremmana) and tried to discriminate between two muscles in the different breed groups through the use of chemometric tools such as principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), and orthogonal projections to latent structures discriminant analysis (OPLS-DA) (Ritota et al., 2012). It was possible to identify two muscle origins, namely Buffalo and Chianina, and the use of VIP (variable importance in projection) values allowed to determine the most relevant metabolites for these classifications. Genetics plays also an important role in the selection of specific breeds with improved growth rates and, in the case of poultry, breast yield, since these factors will also increase commercial meat production. These advances, though, can be associated with various alterations in meat quality. For chickens, the genetic improvements related to the rapid and large growth of birds, result also in histological and biochemical modifications of muscle tissues that lead to different types of myopathies (Mudalal et al., 2015). The increased rate of muscle growth, at a rate which is greater than the physiologically sustainable one, leads to muscle damage and myodegeneration. Breast muscle fibers of the fast-growing strains are characterized by a shift toward glycolytic metabolism. In addition, compared to the unselected breeds, they have an increased fiber diameter and intercapillary distance, a decreased capillary-to-fiber ratio and rate of protein degradation (Mudalal et al., 2015). Due to these changes, some abnormalities related to structure and metabolism have appeared and are usually coupled

to muscle myodegeneration and regeneration (Petracci and Cavani, 2012). The main abnormalities observed are the so-called "white striping" (Kuttappan et al., 2009) and "wooden breast" (Sihvo et al., 2014). White-striping of breast fillets is an abnormality characterized by the presence of white striations parallel to muscle fibers on the surface of the Pectoralis major muscle and affects severely approximately 3% of Italian medium-sized birds (average live weight 2.75 kg) from both standard and high-growth genotypes raised under commercial conditions (Petracci et al., 2013). This abnormality can also be accompanied by the wooden breast defect, which affects the fillet with a visible hard, bulging, and pale area in the caudal part (Sihvo et al., 2014). These histological abnormalities affect the esthetic characteristics of meat which are strongly impaired for consumer acceptance and the product is consequently downgraded by the poultry industry. Besides the detrimental consequence on the sensorial quality, some studies have shown that meat affected with white-striping also has inferior technological properties, like a poor texture and a decrease in the binding and WHC, and is then used for the production of processed meat products with mixing with other amending ingredients (Petracci et al., 2013). It is thus important to investigate the quality of these meat types and classify the production according to their composition, in order to decide the best processing chain better valorizing the product. A study by Bertram et al. has recently investigated the molecular profile of wooden breast fillets employing metabolomics based on HR-MAS proton NMR spectroscopy (Sundekilde et al., 2017). Different experimental conditions for spectra acquisition have been adopted, namely one-dimensional nuclear Overhauser spectroscopy (NOESY) and Carr−Purcell−Meiboom−Gill (CPMG) pulse sequences with two different echo times (50 and 400 MS), in order to enable the best characterization of the molecular profile of the chicken muscle tissue. Multivariate data analysis proved that the muscle affected by the abnormality had lower concentrations of anserine, carnosine, and creatine compared with normal muscles. Since creatine is an important energy reservoir in muscle tissue, while anserine and carnosine play a role in homeostasis of muscles, these variations imply alterations in the buffering and antioxidative capacities of the muscle. In addition, lipid fraction was higher in both NOESY and CPMG spectra of wooden breast muscles. Because the same result emerges within both experimental conditions, it is proven that the occurred modification is consistent with an increased amount of intracellular lipids. All this information is clearly very useful for the understanding the underlying molecular mechanisms that are involved in this abnormality, and prove that these metabolomics techniques can provide early biomarkers for the definition of chicken quality, especially as a tool to drive the newest challenges in the selection of poultry destined for the production of meat with quality attributes more satisfactory for consumers and industries.

9.1.3.2 Rearing Conditions/Origin of Meat

Geographical origin of meat has been increasingly considered by consumers and producers both for safety reasons (i.e., bovine spongiform encephalopathy (BSE) issues) and for quality standards (Certified Origin Products and specific rearing conditions such as organic). Normally, this determination was carried out through the analysis of stable isotopes ratios, though NMR has recently started to be employed in this assessment, due to its ability to determine rapidly the whole molecular profile of a food product. Again, there are no current applications reported in literature for poultry meat but the knowledge emerging from metabolomics studies applied on meat of different origin could be easily foreseen as applicable also to poultry. Jung et al. employed 1H-NMR spectroscopy and multivariate data analysis on extracts of beef originated from four different countries (Australia, Korea, New Zealand, and USA) in order to highlight the differences in the metabolite profile of raw beef depending on its origin (Jung et al., 2010). PCA and OPLS-DA showed significant differences among the four countries; in particular OPLS-DA showed that the metabolites mostly responsible for this separation were succinate and amino acids such as isoleucine, leucine, methionine, tyrosine, and valine. A one-way analysis of variance (ANOVA) was also employed to further validate these results. Thus, metabolomics based on NMR provided links between composition and the geographical origin of a meat product, although other factors must be kept in mind in the evaluation of the results: in this case, for example, succinate is widely used as a substitute for salt in feedings and therefore the differences in this metabolite might be due to different dietary regimens. Another aspect that might influence meat quality is the animal welfare standards followed within the production systems. The high stocking density during farming might in effect cause various levels of stress in the animals, influencing the final characteristics of meat. The presence of biomarkers in meat related to animal stress may be considered as a further indicator of origin due to specific production standards defined by regulations adopted by producers' consortia, based on specific territories, which could differentiate some farms from others. The commercial value of best practice for animal welfare is not negligible, as modern consumers tend to prefer products with a certificated production respecting animal welfare. It is thus important to have scientific and objective tools to assess indicators of animal welfare to be put in relation to the farm originating the meat. Marco-Ramell et al. investigated serum proteome from three groups of cows subjected to three ranges of welfare: good, semiferal conditions, and hardest conditions (in the mountains) (Marco-Ramell et al., 2012). Difference gel electrophoresis (DIGE) labeling of serum, followed by two-dimensional electrophoresis and MALDI-MS or ion trap MS was carried out to demonstrate that the proteins responsible for discrimination among the three groups belonged to three main pathways: the oxidative

stress (glutathione peroxidase and paraoxonase), the acute phase protein family (Heremans Schmid glycoprotein alpha2), and the complement system. Other biomarkers of the redox status such as superoxide dismutase and glutathione reductase were altered depending on the rearing conditions of the animals. These results show that the hardest farming conditions, with feed restriction and less human care and contact, will induce increased levels of carbonyl content in plasma proteins and a greater activity of superoxide dismutase and glutathione peroxidase, meaning that animals are at higher levels of oxidative stress. This study also proved that biomarkers for animal welfare and stress can be selected for classification, such as glutathione peroxidase, Heremans Schmid glycoprotein alpha2, and cholesterol and fecal corticosterone.

9.1.3.3 Feed

As already stated, diet can impact on the metabolic profile of an organism in two ways: feed molecules become host metabolites once digested and adsorbed by the animal, and they modulate gene transcription by epigenetics modifications. In fact, feed components might directly result in meat metabolites and thus influence the characteristics of meat such as flavor or color, oxidition stability, or nutritional value. In other cases, the specific nutrition could give rise to modifications in the biological performance through epigenetic modulation of the genetic traits.

In 2015, Watanabe et al. investigated the relationship between feeds and glutamate, the most taste-active component of meat (Watanabe et al., 2015). Broiler chicks of 14 days were fed two different diets for 10 days: one containing 100% of the recommended lysine content (precursor for glutamate) and one containing 150% of the recommended lysine intake. The concentration of free amino acids were measured in muscle tissue through the employment of CE-TOFMS. It was found that, of the 127 metabolites identified, 68 were upregulated and 22 downregulated in the group on the diet with a higher content of lysine: more specifically, the products from the degradation of lysine, saccharopine, alfa-aminoadipic, and pipecolid acid were greatly increased in this group. In addition, the muscular content of free glutamate in the 150% lysine diet group was increased by 44% in comparison to the control group. The epigenetic mechanism of such an effect was further validated by the measurement of the mRNA level of the relative enzymes. These results show that all the products from lysine degradation are increased when lysine content is increased in the diet and the content of free glutamate is also regulated by this degradation, showing that a higher lysine diet can improve the taste of meat. Another key characteristic in modern meat quality is leanness and this trait could be easily associated to the animal diet. Health awareness among the consumers has been increased, consequently directing their preference toward lean meat. The latter may be produced by overdosing feed additives such as L-carnitine, ractopamine, nucleotides, due to their

lipolytic activity and better effect on growth performance of birds (Zuo et al., 2010; Yasmeen et al., 2013). Costa-Lima et al. investigated the effect of ractopamine on the sarcoplasmic proteome of Longissimus thoracis pork muscle (Costa-Lima et al., 2015). The relative proteome was analyzed through two-dimensional electrophoresis and MS and nine spots were found to be different between the treated and the control group and these were correlated to glycolytic enzymes. In this way they proved how ractopamine had an effect, not only by perturbing lipolysis, but also by affecting the proteome, thus influencing the conversion of muscle to meat. Another important aspect of feed is related to the consumers' perception that domestic meat is believed to be of higher quality owing to the feeds employed for its production (Henchion et al., 2014). It is therefore important to develop specific methods to determine the link between product quality and feeding, in order to scientifically substantiate promotion of local meat consumption only when appropriate. Local productions, besides, usually focus on specific characteristics for the meat product, which are recognized and appreciated by the consumers, and studies have been focused on the development of specific feeds for selected animals enhancing the esteemed sensorial attributes, therefore generating added value products. Sánchez del Pulgar et al. identified, through PTR-ToF-MS, the metabolite profile of lean and subcutaneus fat from dry-cured Iberian ham in order to identify possible differences due to different diets (Sánchez del Pulgar et al., 2013). Pigs fattened outdoors with pasture and acorn or pigs fed with a high-oleic concentrated feed were compared by application of univariate (ANOVA) and multivariate data analysis (PCA and PDA, Penalized Discriminant Analysis) of mass spectra. Samples were successfully discriminated in relation to the feed employed: the first group, fed on acorn and pasture, showed higher concentrations of ketones and aldehydes, probably due to the greater amount of fatty acids in the feed, and lower contents of sulfur-containing compounds. Again, the metabolomics approach proved useful for this kind of quality assessment: the method employed was very rapid and might be employed in the future for non-destructive online monitoring of quality. Jurado et al. also studied Iberian pigs after two different diets: in this case "extensive" and "intensive" fattening systems, and focused on the use of 4-methylsterols and 4,4-dimethylsterols as biomarkers (Jurado et al., 2013). GC-MS and GC-flame ionization detector (FID) were employed to identify and quantify the compounds in the samples and, in this case, six methylsterols were determined and described for the first time in these samples. These metabolites were analyzed with pattern-recognition techniques, such as PCA and linear discriminant analysis (LDA), in order to discriminate between the two diets. In the end, a multilayer perceptron artificial neural network was able to differentiate between the two systems with a 91.7% classification performance. The importance of feeding as determinant for the consumers' choices is also attributable to the request of locality, i.e., very-short supply chains for animal production,

because the far industrially produced fodders are negatively perceived in comparison to local crops. Zancanaro et al. employed NMR spectroscopy to discriminate between muscles of pigs fed with either an industrial feed or a 0-miles feed with a similar composition (Zancanaro et al., 2011). Lipidomics showed that the molar percentage of 18C fatty acids and the mean fatty acid chain length were different between groups. PCA was also applied on the NMR spectra and showed some discriminating potential markers between the two groups due to phosphorylated compounds and some amino acids. Again, NMR metabolomics has not yet been employed in the poultry sector, but the experimental design adopted in the above studies can be usefully employed in the assessment of the effect of external factors such as feed on poultry meat quality. Investigation on meat quality in relation to feed could be also correlated to the importance of tackling food fraud. This is of particular importance when frauds also have implications for food safety. After food scares such as BSE, a 2002 EU regulation prohibited the use of animal by-products and processed products of certain animal species for the purpose of feeding the same species (Regulation (EC) No 1774/2002). Nonetheless, feeds with rendered animal protein from parts and bodies of animals of the same species might be still employed fraudulently, due to their extreme cheapness. Therefore, such a fraud might also represent a safety issue. Most recently, therefore, many studies investigating this issue were carried out. Unfortunately, only a few of them were addressed to poultry, although animal by-products, e.g., protein hydrolysates, are explored as ingredients for their feeds, in an effort to promote sustainable systems which more and more require the exploitation and valorization of waste. For instance, a purified protein hydrolysate with zero chromium from chrome-tanned leather waste has been tested as poultry feed (Chaudhary and Pati, 2016). The study produced evidence that protein hydrolysate can replace up to 75% of soybean meal in broiler diets without affecting either growth performance or meat characteristics. However, the traditional analyses employed to evaluate the meat quality, e.g., ultimate muscle pH, color, protein, fat, and mineral content, are far away from the holistic view provided by the omics approach and a definitive response for the equivalence of such feeding has not yet been reached. In another study, Cajka et al. investigated the possible differences in chicken meat in relation to a feed with (5%−8% w/w) and without the addition of chicken bone meal (banned component) employing DART-MS and multivariate data analysis both on the feed and on chicken muscle (Cajka et al., 2013). The metabolite fingerprint was assessed for both feed and muscle extracts and these were evaluated through PCA and OPLS-DA. In this way it was possible to discriminate between the two diets on polar and nonpolar extracts, and this was assessed positively also after 6 months. One thing that was questioned, though, was that triglycerides (in nonpolar extract) showed pattern variation due also to season and other raw materials used in feed preparation, thus complicating the assessment of the effect of the chicken bone meal.

9.1.3.4 Pre- and Post-slaughter Conditions

Some of the main defects in poultry meat are the so-called pale, soft, exudative (PSE) and dark, firm, dry (DFD) meat. These defects involve meat color, mainly, and the texture, with detrimental effects provoking consumers to discard the affected meat. PSE and DFD are due to unusual postmortem pH evolution in meat: very low pH in PSE meat and high pH in DFD meat are reached ultimately, giving higher chances of microbial growth. These defects are caused by stress-related shocks in the animals before death, causing alterations in their metabolism and thus postmortem variation in meat. For PSE meat, genetic links were found in pigs, while in chicken this defect is still mainly related to preslaughter stress and inadequate meat chilling. In any case, these factors will cause either a fast postmortem glycolysis and thus a fast pH descent or just abnormally low values of pH for the meat. In DFD meats, instead, prolonged stress will cause the use of glycogen supplies and thus, postmortem, the absence of lactate will cause higher pH values. Many tests are now available to assess these two types of defects; in PSE genetic tests are also possible for swine, though most recently metabolomics was proposed as a rapid alternative. In poultry, metabolomics will be essential since genetic links are still not evident. However, publications concerning studies for PSE and DFD signatures based on metabolomics are still absent from the literature for birds and, again, the potentiality of this approach will be described below for pork. NMR was used to study the possible consequences of preslaughter exercise on the metabolic profile of plasma, employing 40 pigs divided into different groups: control group and exercise on a treadmill followed by 0, 1, or 3 h of rest preslaughter (Bertram et al., 2010). It was seen that plasma lactate greatly increased in the group with no rest between exercise and slaughter, while when rest was present, the stress-related variation in the metabolic profile were attenuated. It was found that plasma lactate was greatly correlated with WHC, muscle temperature 1 min postmortem and meat pH. In this way, it was possible to see that even without directly measuring the whole metabolic profile of meat, it is possible to evaluate meat quality through a specific metabolite content in preslaughtering animals and adopt it as the marker for predicting the impact of stress. The same research group employed 31P-NMR spectroscopy to investigate the phosphorylated metabolome in an attempt to determine whether two different postmortem cooling profiles, commercial batch (soft) and tunnel chilling (fast), would generate differences in pork meat quality (Bertram et al., 2001). Phosphocreatine was degraded less rapidly postmortem in tunnel chilling, while ATP showed no degradation differences in the two cooling methods. pH values also differed, with a maximum variation between groups of 0.25 units after 150 min postmortem. It was also calculated that the fraction of inactivated myosin was 2.5 times greater using the soft method, indicating that the measured differences in pH and temperature

among the two regimes could influence protein degradation and WHC of meat. These studies show the great potential of metabolomics as a rapid tool to assess the presence of meat defects and it could be easily employed on chicken meat, too, in order to spot the defects in time and to avoid including PSE/DFD meats in the formulation of products which they are not suitable for. In effect, PSE meat could be used in special products formulated in order to restore a proper color and protein functionality, so as to improve the texture and contain yield loss, while DFD meat cuts, due to their microbiological instability, could be used in heat-processed products (Lesiów and Kijowski, 2003). It is clear that metabolome perturbations are mirrors of a more general alteration in the muscle physiology, reflected also at the level of the proteome characterizing the muscular apparatus, as affected by different enzymatic activities, e.g., by hydrolases. Zanetti et al. investigated the proteome of chicken *Pectoralis major* muscle in order to evaluate possible changes due to transit time before slaughtering (90 or 220 min) (Zanetti et al., 2013). Two-dimensional electrophoresis, followed by image analysis and LC-MS/MS of the points of interest, chosen by means of statistical analysis (Wilcoxon's test and PCA) were applied as blood tests. They showed that the animals with longer transit times had higher stress levels as evidenced by the presence of two spots related to time in transit, associated with proteins linked to cellular housekeeping functions (metabolism, cell division, apoptosis control). PCA did not show a clear separation in the proteome of chicken with different in-transit times, though some small differences were identified. For future studies, thus, employing more different times and other tissues could be more helpful in gaining insight into the stress-related proteome changes. It was clear, though, how this preslaughtering process can hinder good meat quality by causing stress to the birds. Another important factor affecting meat quality is the slaughtering procedure itself. Salwani et al. investigated the differences in the proteome of the skeletal muscle of broiler chickens with or without gas stunning prior to slaughtering (Salwani et al., 2015). Meat quality parameters (color, pH, shear-force, and drip/cooking-loss) were also measured and differences were found between the two treatments. Gas-stunned chickens had lower redness, pH, and shear-force, while they showed greater drip and cooking loss both at 4 and 24 h postmortem. In addition, proteomics, through two-dimensional PAGE on samples at 7 min postmortem, showed that gas-stunning upregulated the expression of creatine and pyruvate kinase and beta-enolase. These results show that pre-slaughtering treatments having negative effects on meat quality (WHC and color, especially) and provoke increased speed of the postmortem energy metabolism. Most recently, Beauclercq and colleagues (Beauclercq et al., 2016), employed high-resolution NMR (1H and 31P) in order to investigate the actual variation in chicken *P. major* muscle and serum due to the different values of meat ultimate pH, a characteristic that, as mentioned above, is fundamental for the quality of poultry meat and

related to DFD/PSE meats. Two genetic lines of chickens with either high or low ultimate pH (pHu + or pHu −) were selected: 20 male broilers were chosen from the first one and 19 from the second one. The pHu + line showed a significantly lower glycolytic potential and was darker and less exudative. OPLS-DA was carried out on the concentration of spectral metabolites and a total of 26 molecules (from 1H and 1P spectra) were found to be discriminative in muscles and 20 (just from 1H spectra) in serum. The pHu − line was found to have higher carbohydrate content in both muscle and serum, together with other metabolites linked to production of energy. On the other hand, the pHu + line, showed a metabolism more switched toward ketogenic amino acid degradation and lipid beta-oxidation, since less glucose and glycogen are present and thus amino acids such as alanine are employed for energy production. This study thus paved the way into the clearing of the mechanisms behind the different glycogen levels at slaughter (and thus different quality traits) and suggested a fast screening technique to exclude meat cuts of poor quality, by employing a defined set of spectral metabolites to exclude pHu + cuts.

9.1.3.5 Meat Processing

Modern consumers require meat products that are both convenient and of high quality, possibly appearing as close as possible to minimally processed foods. Thus, it is important for the meat producers to develop new technologies capable of providing these products, while maintaining good preservation of the quality attributes. Whatever the processing technology applied, food composition and structure evolve during production and storage, and the main purpose of the industry is to take control over meat transformation processes, by monitoring opportune markers of quality. During a processing step such as ripening, for example, the peptidic profile of a food product changes greatly and can give hints on the origin and the evolution of the specific protein fraction. In effect, physical, chemical, and enzymatic processes occur during ripening and modify the food matrix. Reactions such as proteolysis, lipolysis, and fermentation (i.e., lactic and propionic), change the chemical components of the food product, impacting on its final sensory properties.

Flavor, for example, is greatly influenced by proteolysis, which could contribute both positively (with the release of specific amino acids and peptides), and negatively, with the production of off-flavors (such as from bitter, hydrophobic peptides). Moreover, it could liberate substrates used in other reactions. Therefore, it is fundamental to gain the desired, balanced, breakdown of proteins into specific amino acids and peptides, in order to obtain an acceptable flavor for that food product (Mamone et al., 2013). Poultry, especially turkey, is increasingly sold as a cured product to extend shelf-life and increase consumers' acceptance. Thus, the experience gained so far in other more consolidated meat-curing industries, including adoption of omics

approaches for quality control, is of inspiration for developing analogous systems also in less traditional sectors, such as that of cured poultry meat.

Proteomics has investigated the biochemical changes occurring in dry-cured ham during its processing: during the postmortem phase, endogenous proteolytic enzymes are activated and this induces the degradation of muscle proteins (Mamone et al., 2013). In fact, muscle sarcoplasmic proteins have been found to be relevant substrates for proteolysis by the identification of many small peptides, which are released by enzymes from the glycolytic pathway (Mora et al., 2011a).

Due to the fact that there is a lack of cleavage specificity, it is usually hard to identify the peptides released in food products. In spite of this, information on the endogenous proteases both from early stages (calpains) and medium- and long-term (cathepsins), and their actions, can be gained through the employment of multistage MS-based peptide sequencing (Mamone et al., 2013).

Mora et al. (2009) have demonstrated in dry-curing hams that there is an additional intense secondary proteolysis from amino- and carboxy-peptidases. This was assessed through the identification of oligopeptides with consecutive loss of terminal amino-acids.

Moreover, both small and large fragments of heavy and light chains of myosin and actin were identified through MS methods in dry-cured ham, proving that muscle myofibrillar proteins also experience extensive proteolysis (Di Luccia et al., 2005; Sentandreu et al., 2007; Mora et al., 2011b). In fermented sausages, it was studied how lactic acid bacteria cause an even greater proteolysis (Picariello et al., 2006). Nonetheless, only a few studies regarding these products have been published in the field of peptidomics, and it could be interesting to employ this approach to define the specific proteolytic patterns in dry fermented sausages in order to assess, or even predict, the stability of determined strains of starter cultures of lactic acid bacteria (Mamone et al., 2013).

Recently, Sentandreu and Sentandreu (2011) proposed MS for the identification of species-specific biomarkers in order to determine the authenticity of meat-derived products.

Moreover, MS analysis allowed the characterization of the domains of food proteins stable to digestion. In cured beef Bresaola, which is extensively hydrolyzed by endogenous proteases, a large variety of peptides are released from the early postmortem phase onwards. Many of these peptides can exert several biological activities in the human body (Ferranti et al., 2014). After ingestion, the proteins, polypeptides, and peptides from Bresaola are further degraded by the proteases found in the gastrointestinal (GI) tract. Ferranti and colleagues (Ferranti et al., 2014) have employed a static in vitro digestion model, including oral, gastric, and duodenal phases, to evaluate the effects of different curing protocols on the digestibility of Bresaola meat. The obtained digestates were profiled by MS-based proteomic and peptidomics strategies (Ferranti et al., 2014).

It was seen that, at the end of digestion, all samples were almost identical for the majority of the released peptides. Only some slight differences, due, possibly, to differing technological procedures or nature of the raw material, were visible. The only thing that could be associated with the characteristics of the food was the kinetics of the peptide release, thus the evolution in time of the quantitative composition of the profile of the digestates (digestome).

In this case, sarcoplasmic proteins were degraded relatively quickly, while the myofibrillar chains would not be completely hydrolyzed by the action of gastro-duodenal proteases, even after a prolonged hydrolysis. Moreover, these chains require a previous step of proteolytic release (Ferranti et al., 2014). This study identified more than 170 peptides, which were liberated from both sarcoplasmic and structural (actin and myosin) muscle proteins. Of note, many of the identified peptides are considered precursors of sequences which are potentially antioxidant and antihypertensive (Ferranti et al., 2014).

Bordoni et al. also investigated Bresaola samples through the metabolomics approach (Bordoni et al., 2014). Samples were again digested in vitro and digestates were investigated through 1H-NMR to study the influence of the matrix in the bioavailability of nutrients. In effect, the meat matrix has been shown to modulate the free diffusion of specific nutrients, e.g., carnosine, in the digestion media toward the absorption sites, thus affecting the nutritional quality of the product. The developed protocol is applicable for screening of the nutritional value of protein-rich foods, being able to assess the bio-accessibility of protein end-products.

9.1.3.6 Product Formulation

The specific formulation of a meat product is important both for sensory and nutritional quality and for its technological characteristics, though in some cases it is important to determine it to avoid fraudulent practices. A study from Lassoued et al. proved, for chicken meat, that there is a link between risk aversion and ethically motivated behavior on one side, and the trust the consumers have in a brand and in the declared quality attributes on the other side (Lassoued et al., 2015). It is therefore important for food industries to build trusting relationships with consumers by providing scientific evidence, i.e., objective descriptors, to discriminate between different products in terms of their quality attributes. On the other hand, the promotion of frauds is stimulated because producers are facing a very competitive global market, thus they need to find the cheapest way to produce their meat bypassing the quality-check indicators. It is therefore necessary to find new scientific methodologies to reinforce the quality-check system and to fight food frauds, as also mentioned in the case of feeds. One of the main ingredients employed in the formulation of meat products is mechanically recovered meat (MRM). This ingredient is industrially obtained by recovering residual

meat from animal bones or poultry carcasses from which the bulk of the meat has already been removed. MRM has the appearance of finely comminuted meat and is incorporated in a wide range of meat products, as a cheap source of meat. Similarly to MRM, desinewed meat (DSM) is produced using a low-pressure technique to remove meat from animal bones.

MRM and DSM meat are similar to hand de-boned meat (HDM) in their chemical composition, though they have a lower commercial value (Mamone et al., 2013). Moreover, they have been excluded from the EU definition of meat with the Directive of the European Parliament N. 101/2001. This was also due to the fact that consumers were concerned about their safety (Mamone et al., 2013).

It is thus necessary to discriminate between MRM and DSM, and HDM with reliable analytical methods. Recently, Surowiec and colleagues (Surowiec et al., 2011a) carried out a proteomic study aiming at identifying potential markers capable of detecting chicken from MRM. In this research, proteins were extracted intact from raw meat and analyzed by the employment of OFF-GEL electrophoresis, sodium dodecyl sulfate (SDS)-PAGE and, finally, nano-LC-MS/MS to identify the markers. Researchers were thus able to define the key proteins from the processed meat samples and identify the specific markers for MRM chicken (Surowiec et al., 2011a; Mamone et al., 2013). Surowiec et al. also employed metabolomics techniques for the same kind of assessment on HDM pork and chicken meat, MRM meat and DSM meat samples of different origin (Surowiec et al., 2011b). In this case, GC-MS was used to determine the molecular profiles of samples and chemometric tools (PCA and OPLS-DA) were employed for the identification of specific differences between types of meat. It was found that the supervised statistical technique (OPLS-DA) was capable of separating all classes of meat-products (HDM, MRM, DSM) for both pork and chicken. The metabolites that generated this separation were then confirmed by Student's *t*-test and were tentatively identified by comparison with specific databases. However, these metabolites appeared to belong to numerous chemical classes and were mostly fat-related, thus, currently, none could serve as a specific biomarker, but future research could help in this assessment, in order to develop a fast methodology for the identification of HDM, MRM, and DSM meats. The blending of meat from different species in a food product might be considered fraud when not declared, and can be a relevant character of quality control for determined consumers, such as the ones concerned with ethical aspects (i.e., against the use of horse meat) or religious requirements (e.g., no pork consumption). Recently, also, some food scandals occurred in Europe with horse-meat contamination of "beef" lasagna and traces of pork in Muslim school meals of chicken in the United Kingdom (D'Alessandro and Zolla, 2013). It is necessary, therefore, to be able to identify these frauds in order to protect consumers. Von Bargen et al. employed MS in order to detect trace contamination of pork and horse meat and to determine the

specific biomarkers of these two types of meat against beef, chicken, and lamb (von Bargen et al., 2013). Through a proteomic approach, the typical biomarker peptides of the five different species were identified and these were taken into consideration in the study. Researchers were able to detect 0.55% horse contamination and 0.13% of pork contamination in beef matrices, showing that this proteomics technique can be of great help in the assessment of fraud. A fast and sensitive method, based on multiple reaction monitoring, was developed and this can be applied in a routine manner in quality-control laboratories employing tripe-quadrupole MS instruments, representing an alternative to genomics and classical proteomics techniques that are more expensive (i.e., ELISA) or still problematic for this type of assessment (i.e., PCR).

Sentandreu et al. (2010) employed a proteomic approach capable of detecting chicken meat in mixed meat preparations. In this study, myofibrillar proteins were extracted, the OFFGEL isoelectric focusing was used to enrich target proteins, then myosin light chain 3 was digested through in-solution trypsin digestion. Finally, LC-MS/MS was employed to analyze the peptides thus generated. Through this method, researchers were capable of detecting, with a high confidence, 0.5% of contaminated chicken in pork meat. AQUA stable isotope peptides, synthesized using sequences of species-specific peptide biomarkers, which were selected previously, allowed the quantitative detection of chicken meat. There was a linearity between the amount of chicken in the meat mixture and the amount of peptide biomarkers found. The method now needs to be validated by other independent replications, though it proved to be very simple and effective. This method could be used with both cooked and raw meat. It could also be adapted and employed for highly processed food, where immunoassays are precluded due to the modifications occurring in the tertiary protein structure during processing.

Moreover, the proteomic analysis employed in this study was capable of identifying a definitive discriminatory sequence of peptides. The traditional DNA and PCR-based methods do not allow this. The researchers have also proposed and expect future applications of this method as a regular procedure, by translating this technology into routine MS instruments. This will be very useful in laboratories used for public inspections (Sentandreu et al., 2010; Mamone et al., 2013). Another important application in the determination of specific food ingredients in a formulation is in the case of food allergens, such as soy.

Soy proteins can be added as emulsifiers in meat-derived proteins, to work during heat treatment. Their use, though, could be limited or forbidden by specific regulatory authorities in some countries. Suitable analytical methods, capable of detecting and/or quantifying soybean proteins (soy protein isolate (SPI)) in meat products are thus needed, so that it will be possible to assess whether the threshold levels for their contents are respected (Mamone et al., 2013).

Currently, only a few methods are available for their semi-quantitative determination. Authorities such as the FDA (USA Food and Drug Administration) have criticized the Official Method (AOAC Official Method of Analysis 988.10), which is based on an ELISA assay. This method is, in fact, not considered too reliable or accurate (Mamone et al., 2013). Moreover, methods capable of detecting even low ppm concentrations of soybean proteins are needed, since, as stated, these molecules have an allergenic potential (Koppelman et al., 2004; Mamone et al., 2013).

The alternative to the traditional method, are LC-based assays, but these could only work with raw meats. Recently, a proteomic study (Leitner et al., 2006) aimed at identifying SPI adulteration in heat-processed meat products from different origins (chicken, turkey, pork, beef), employed multidimensional HPLC-MS/MS. In this research, in order to isolate the interesting peaks originating from SPI-containing meat products, it was necessary to pre-fractionate samples through the employment of perfusion chromatography. The fractions were then digested using trypsin and finally analyzed by nanoflow LC-MS/MS.

Glycinin and conglycinin (major seed proteins), in many variants and subunits, were spotted in SPI samples, together with other two proteins. Meat samples containing SPI were characterized by peaks from different glycinin A subunits. The subunit A4 from glycinin G4 was in fact found in all SPI samples and could be used for new analytical methods for the identification of SPI in processed meat (Leitner et al., 2006; Mamone et al., 2013).

9.1.4 Conclusions and Future Applications

Proteomics and metabolomics techniques and their associated methodologies have helped in many research fields to find interactions between genome and external factors able to modify the characteristics of a biological system. The three approaches have been increasingly employed also in food science and in the assessment of meat quality. As stated, the quality of meat products depends on many different characteristics that, in turn, are influenced by genetics, but also by various external factors, such as the rearing methods and feeds employed, the handling of slaughtering and the processing and formulation of meat-based products (Table 9.2).

Many reported studies were based on cattle or swine, although the same principles stand and could be applied to chicken meat. This is especially the case for the investigation of the occurrence of PSE and DFD meat. In this sense, the employment of conjoint genomics, proteomics, and metabolomics studies could help to provide a complete insight on these defects that majorly impact chicken meat. In addition, these techniques could be helpful in the investigation of other defects such as white striping or wooden breast. The development of fast methodologies that could be applied online would be of great help in the determination of the destination of specific meat cuts

TABLE 9.2 Reported Proteomics and Metabolomics Studies on the Factors That Impact on Meat Quality

	Proteomics	Metabolomics
Breed/genetics	Mazzeo et al. (2008)	Straadt et al. (2011)
	Piñeiro et al. (2003)	Ritota et al. (2012)
		Sundekilde et al. (2017)
Rearing condition/origin	Marco-Ramell et al. (2012)	Jung et al. (2010)
Feed	Costa-Lima et al. (2015)	Watanabe et al. (2015)
		Sánchez del Pulgar et al. (2013)
		Jurado et al. (2013)
		Zancanaro et al. (2011)
		Cajka et al. (2013)
Transport/preslaughter conditions	Zanetti et al. (2013)	Bertram et al. (2010)
	Salwani et al. (2015)	Beauclercq et al. (2016)
Postslaughter handling/ chilling		Bertram et al. (2001)
Processing	Mora et al. (2011a)	Bordoni et al. (2014)
	Mora et al. (2009)	
	Di Luccia et al. (2005)	
	Sentandreu et al. (2007)	
	Mora et al. (2011b)	
	Picariello et al. (2006)	
	Sentandreu and Sentandreu (2011)	
	Ferranti et al. (2014)	
Additives/formulation	Surowiec et al. (2011a)	Surowiec et al. (2011b)
	Von Bargen et al. (2013)	
	Sentandreu et al. (2010)	
	Leitner et al. (2006)	

(i.e., meat-based products or full cuts). Moreover, in the modern market it is necessary to give consumers as much objective and biomarker-assisted information as possible, and it is thus of great help to have techniques such as proteomics or metabolomics to be able to confirm the quality of a product

and the absence of frauds. This is important, for example, in the determination of the nutritional quality of chicken meat, considered a lean food, with many important bioactives, or for the certification of specific types of rearing (i.e., free range) considering the increasing interest in animal welfare. To conclude, both proteomics and metabolomics will have a great impact on the evaluation of chicken-meat quality and could help breeders and industries to evaluate genetic selection on the quality of the food products in the future.

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Chapter 10

Vibrational Spectroscopy for Quality Assessment of Meat

Ana M. Herrero[1], Pedro C. Hernandez[2], Francisco Jiménez-Colmenero[1] and Claudia R.-C. Perez[1]

[1]Institute of Food Science, Technology and Nutrition (ICTAN-CSIC), Madrid, Spain, [2]Institute of the Structure of Matter (CSIC), Madrid, Spain

10.1 INTRODUCTION

Meat is a rich nutrient matrix with high-value dietary components and generally meat demand and consumption is very high in most countries. Although, during recent years, a modest and decelerating growth in world per capita consumption of meat has been taking place, the world meat economy has been characterized by the rapid growth of the poultry sector (FAO, 2012). In fact, in developing countries, where almost all world population increases take place, it is estimated that meat consumption will increase considerably to 9 kg in 2030 and to 18 kg in 2050, of which 12.5 kg will be poultry meat (FAO, 2012).

Meat is a very heterogeneous product since the chemical composition, technological and sensory attributes are highly influenced by several factors. Meat quality is a result of complex interactions between the biological traits of the live animal and biochemical processes that occur postmortem as muscle is converted to meat, preserved and consumed (Jimenez-Colmenero et al., 2016). The variability in meat quality stems from several factors (including those relating to species) that arise throughout the production chain; therefore, it is the main concern of the industry (producers and manufacturers), distributors, retailers, consumers, and authorities. In this context, interest is centered on establishing the most effective methods of assessing the quality of meat and meat products. Traditionally, for evaluation of meat quality, sensory and microbiological analysis has been used as well as certain physicochemical methods (texture, water binding properties, color, etc.). Nevertheless, most of these traditional quality methods are time consuming and destructive, and it is not possible to apply them online during handling, processing, and storage. These are the main reasons why, in the last few

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years, great efforts have been made in order to improve the methodology for the measurement of the objective quality of meat, in order to identify methods that solve all the disadvantages of the traditional methodology and may become sufficiently robust, rapid, and nondestructive or noninvasive for the quality assessment of meat. In this respect, the use of vibrational spectroscopic techniques (infrared (IR) and Raman spectroscopy) have several advantages compared to the traditional methods, due to the fact that they are direct, nondestructive or noninvasive, and their application in situ is possible (Damez and Clerjon, 2008; Herrero, 2008a; Prieto et al., 2009; Herrero et al., 2010). This is the reason why vibrational spectroscopic techniques have increased their importance in the determination of meat quality. Therefore, the purpose of this chapter is to provide insights into the study of structural features of meat components such proteins, lipids, and water using vibrational spectroscopic techniques. Additionally, the aim of this chapter is to cover aspects of the applications of these spectroscopic techniques (IR and Raman spectroscopy) as tools for meat quality assessment.

10.2 STRUCTURAL CHARACTERISTICS OF PROTEINS, LIPIDS, AND WATER DETERMINED BY VIBRATIONAL SPECTROSCOPY

10.2.1 Basic Spectroscopic Concepts

Vibrational spectroscopy, which includes IR and Raman spectroscopies, is based on the transitions between quantized vibrational energy states of molecules. In IR spectroscopy, the energy for these transitions is provided by radiation in the IR regions (near-IR and mid-IR) of the electromagnetic spectrum (Schrader, 1995). Transmission is the most powerful method that is well-suited to liquids and gases, and reflection spectroscopy offers the alternative that is almost always used in meat science. A remarkable increase of mid-IR applications has been produced as a result of the development of mid-IR Fourier transform (FT-IR) spectrometers in conjunction with sampling techniques including attenuated total reflection (ATR), for solids, semisolids, and liquids, due to the advantages offered (Li-Chan, 2010). Additionally, FT-IR microscopes have opened up new applications for in situ microspectroscopic mapping and imaging of food (Sedman et al., 2010). In recent years, the hyperspectral imaging (HSI) technique has emerged as a smart and promising analytical tool for quality-evaluation purposes and has attracted much interest in the nondestructive analysis of different food products. The main inducement for developing the HSI system is to integrate both spectroscopy (mainly near-IR spectroscopy (NIR)) and imaging techniques in one system to make direct identifications of different components and their spatial distributions in the tested products (ElMasry et al., 2012a, 2012b; Xiong et al., 2015). By combining spatial and spectral details, HSI

has proved to be a promising technology for objective meat quality evaluation.

In Raman spectroscopy, samples are excited with a source of monochromatic incident radiation that may be in the ultraviolet (UV), visible (VIS), or NIR regions of the electromagnetic spectrum (Schrader, 1995). In food, visible Raman radiation can be masked by fluorescence. However, Fourier transform Raman spectroscopy (FT-Raman), using NIR excitation from a Nd: YAG laser at 1064 nm, can generally avoid the problem of fluorescence (Li-Chan, 2010). Techniques such as surface-enhanced Raman spectroscopy (SERS), confocal Raman microspectroscopy, and Raman imaging spectroscopy are recognized for their potential and specific advantages in studying food components at very low concentrations, and for in situ multicomponent analysis.

Complementary information on fundamental vibrational modes can be obtained from mid-IR and Raman spectra, as some vibrational motions are detected primarily with IR radiation and others primarily by Raman scattering. Vibrational spectroscopy in particular offers many advantages in food studies (Li-Chan, 1996; Herrero, 2008a,b; Damez and Clerjon, 2008; Cozzolino, 2015; Cheng and Sun, 2015; Santos et al., 2015). Raman and IR spectroscopy can be applied to condensed-phase samples in various physical states, whether liquid or solid, clear or opaque. In many cases, minimal or no sample preparation or pretreatment is required, and a vibrational spectrum can usually be acquired within a relatively short time (Chalmers and Griffiths, 2007).

10.2.2 Spectral Analysis of Complex Biological Systems

Conventional IR and Raman spectroscopies are informative for studying the molecular structure of biological materials and for qualitative and quantitative applications. However, because of the molecular complexity of food which involves band overlapping, it is hard to extract useful detailed information from the spectral data. In order to better identify the individual components and maximize their response, the spectra can be analyzed by two basic techniques, namely, second-derivative formation and self-deconvolution. Second-derivative formation of a spectral contour clearly detects variations in the band components. Self-deconvolution is an artificial resolution enhancement. Both mathematical procedures allow the detection of spectral contributions in an overlapped band spectral profile (Surewicz and Mantsch, 1984). The resulting bands obtained by resolution enhancement (or by second-derivative formation) can subsequently be used to describe the measured spectral envelope by individual band components. These can be used then with aims of quality inspection by using multivariate analysis, e.g., discriminant analysis, receiver-operating characteristic analysis (ROC curves), etc.

10.2.2.1 Two-Dimensional Correlation Spectroscopy

Two-dimensional correlation spectroscopy (2D-COS) is another powerful technique which can be useful for spectral analysis of complex biological systems, such as food (Noda, 2007). This 2D correlation analysis allows the correlation of the dynamic fluctuations of either IR or Raman bands in a series of spectra measured at different times during application of an external perturbation, which can be temperature, pressure, concentration, isotopic exchange, etc.

Cross-correlation analysis provides 2D spectra that are defined by two independent frequencies, ν_1 (abscissa) and ν_2 (ordinate). The synchronous 2D correlation spectra of dynamic spectral intensity variations represent the simultaneous occurrence of coincidental changes in spectral intensities measured at ν_1 and ν_2. Correlation peaks appear at both diagonal (auto-peaks) and off-diagonal peaks (cross-peaks). Cross-peaks, which can either be positive or negative, reflect correlated changes of functional groups within the biomolecular system that occur simultaneously in the same (+) or in the opposite (−) direction. By contrast, the asynchronous 2D correlation representation is characterized by missing autopeaks and asymmetric cross-peaks which reveal uncorrelated (i.e., out-of-phase) behavior of two bands. The time-dependent changes in the spectral intensities are shown as 2D contours, called synchronous and asynchronous maps (S- and A-maps), which correlate to in-phase (synchronized) and out-of-phase (unsynchronized) intensity changes at two frequencies, respectively. Cross-peaks, which occur in both the S- and A-maps, provide information about the temporal order of the spectral changes. Cross-peaks with the same sign in the S- and A-maps indicate that a spectral change of the ν_1 band (abscissa) occurs predominantly before that of the ν_2 (ordinate) band in the sequential order of time. The temporal order is reversed for opposite signs of the S- and A-map cross-peaks.

The advantages of this technique, as compared with 1D vibrational spectroscopy, are summarized as follows: (1) enhancement of apparent spectral resolution of overlapped bands; (2) band assignments through observations of correlations between the bands; (3) studies of inter- and intra-molecular interactions through selective correlation between bands; and (4) probing the specific order in which the intensities of various bands change during application of an external perturbation.

In particular, 2D-COS visible/near-IR analysis has been used to study the thermal processing of chicken meat (Liu et al., 2000). The synchronous map revealed that at least two bands around 445 and 560 nm decreased in intensity with cooking time, which may be assigned to deoxymyoglobin and oxymyoglobin, respectively. The asynchronous 2D-NIR correlation map indicated that C−H fractions were easily oxidized. The effect of cold storage on chicken meat was also studied using generalized 2D visible/NIR correlation spectroscopy (Liu and Chen, 2000). The 2D correlation analysis in the

NIR region indicated that the change in O−H/N−H bands occurred before the change in C−H groups during cold storage. This revealed that during cold storage, water species interacted with other meat components, and this interaction could lead to proteolysis and denaturation of meat proteins, which is associated with the development of tenderization during storage (aging) (Liu and Chen, 2000). From the asynchronous correlation analysis in the visible and NIR regions, the authors concluded that chicken meat discoloration occurred earlier than other developments, such as the tenderization process (Liu and Chen, 2000). Subsequently, the same authors studied the thawing behavior of frozen chicken meat using generalized 2D visible/NIR correlation spectroscopy (Liu and Chen, 2001). The study was carried out when the frozen chicken meat was tightly covered by a quartz window without exposure to air. The synchronous 2D correlation spectrum in the NIR region illustrated that the melting of ice crystals and the relaxation and proteolysis of proteins occurred earlier than the relaxation of lipids.

10.2.3 IR and Raman Spectra

Vibrational spectroscopic techniques (Raman and IR spectroscopies) have proven to be powerful tools in elucidating protein, lipid, carbohydrate, and water structural characteristics, noninvasively and in situ, in food and particularly in meat, (Herrero 2008a,b; Damez and Clerjon, 2008). Because meat is composed mainly of proteins, lipids, and water, Raman and IR spectral regions of these compounds will be analyzed in the following. Changes in the frequencies, half-widths, and intensities of the Raman and IR bands of chemical groups of these compounds are indicative of structural changes. However, since meat is a complex system comprising mainly proteins, lipids and water, analysis of particular meat components should be carried out after previous removal of any spectral influence from the rest of components (see Section 10.2.2).

10.2.3.1 IR Spectra

NIR spectroscopy provides little interpretable protein and lipids structural information because the broad bandwidth leads to severe overlap of most of the bands in the NIR spectra. As a consequence, NIR has not been used for in-depth study of protein and lipid structural changes in foods and particularly in meat. However, near-IR reflectance spectroscopy (NIRS) in combination with chemometric analysis or other techniques such as imaging techniques (called HSI system) has been used in muscle-based foods such as meat to determine the content of its main constituents (Wold and Isaksson, 1997; González-Pérez et al., 2002, Afseth et al., 2005; Khodabux et al., 2007), to distinguish between storage conditions (Thyholt and Isaksson, 1997; Uddin and Okazaki, 2004) and for assessing food quality (Liu et al.,

2003; McGlone et al., 2005; Ripoll et al., 2008; Prieto et al., 2009; ElMasry et al., 2012 a; Qu et al., 2015). Instead, mid-IR spectroscopy can provide information related to protein secondary structure and carbohydrate, water and lipid structure through well-defined IR characteristics bands. The most characteristic bands appearing in the mid-IR spectra of proteins, lipids and water are described below.

Proteins

IR amide I, amide II, and amide III bands should be mentioned as the main bands providing information related to protein secondary structure. In addition, other bands generated by aliphatic and aromatic amino acid residues provide details on protein tertiary structure. Variations in intensity and/or shifting in frequency of these IR bands evidence protein structural changes (Surewicz and Mantsch, 1984; Barth 2007; Herrero et al., 2010). The amide I vibrational mode is found in the 1700- to 1600-cm^{-1} region (Fig. 10.1), with strong intensity, and is mainly due to the carbonyl stretching vibration with a minor contribution from C−N stretching and N−H bending vibrations. This band mostly depends on the secondary structure of protein backbone. In food with high water content, such as meat products, samples have been previously deuterated to avoid water absorption in the protein spectral region (Herrero et al., 2012). In this way vibrational frequencies are usually shifted to lower values. Proteins adopting α-helical conformation have strong

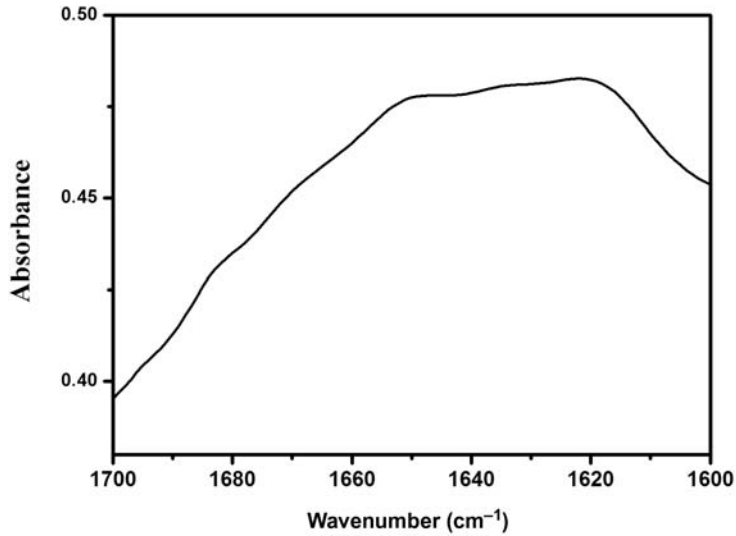


FIGURE 10.1 Typical Fourier transform infrared spectra from a low-fat frankfurter (elaborated with pork meat) in the 1700- to 1600-cm^{-1} region. These spectra were measured from a small portion of deuterated samples using CaF_2 window for transmission infrared spectroscopy.

amide I bands between 1657 and 1650 cm^{-1}, whereas bands between 1640 and 1612 cm^{-1} are commonly associated with β-sheets. The strong water absorption at 1640 cm^{-1} significantly overlaps the amide I band. The amide II band appears in the 1560- to 1510-cm^{-1} range with strong−medium intensity. The other visible amide vibrational mode corresponds to the amide III band which falls in the 1300- to 1200-cm^{-1} region.

Detailed quantitative analysis of amide I and amide II bands is not always an easy task because there is considerable overlapping of a number of bands due to various different secondary structures. The most popular data-processing techniques that avoid overlapping in estimation of protein secondary structures involve either conversion of the spectrum to its second derivative or reduction of the width of the bands by Fourier self-deconvolution of the amide I region to a sum of Lorentzian band components with a nonlinear least-squares procedure (Surewicz and Mantsch, 1984; Herrero et al., 2010, 2012).

Three prominent bands can also be observed in the IR spectra in the 3000- to 2800-cm^{-1} region, which are generated mainly by νCH vibrational modes of amino acids (Surewicz and Mantsch, 1984; Barth, 2007; Herrero et al., 2010).

A strong band at about 3300 cm^{-1} and a somewhat less-strong band at about 3100 cm^{-1} are referred to as the amide A and amide B bands, respectively (Surewicz and Mantsch, 1984; Barth 2007; Herrero et al., 2010). The amide A band is attributed to the νNH vibration, whereas the amide B band is assigned to the first overtone of the amide II vibration, which is intensified by Fermi resonance with the amide A vibration. Changes in these IR bands are associated with changes in secondary structure of proteins.

Lipids

The mid-IR spectra of lipids contain several bands in the 3000- to 1700-cm^{-1} region, and overlapping bans in the 1500- to 700-cm^{-1} region. In these spectra a weak band appears near 3005 cm^{-1} which is generated by the *cis* double-bond CH stretching vibration (νCH =) of lipids. This band is accompanied by methyl group absorption, with characteristic $\nu_s CH_3$ and $\nu_{as} CH_3$ bands appearing as shoulders at approximately 2954 and 2870 cm^{-1}. In addition, the IR region between 3000 and 2500 cm^{-1} is dominated by two strong bands at 2925 and 2854 cm^{-1} (Fig. 10.2) resulting, respectively, from the asymmetric and the symmetric stretching vibrations of the acyl CH_2 groups of lipids (Guillen and Cabo, 1997a,b; van de Voort, Sedman, and Russin, 2001). Modifications of the half-bandwidth of these bands can be generated by changes in lipid chain order/disorder resulting from protein−lipid interactions (Fraile et al., 1999). Narrowing of the spectral profile of these bands (2922 cm^{-1} ($\nu_{as} CH_2$) and 2852 cm^{-1} ($\nu_s CH_2$)) is generally attributed to increasing conformational order of lipid acyl chains (Fraile et al., 1999). In

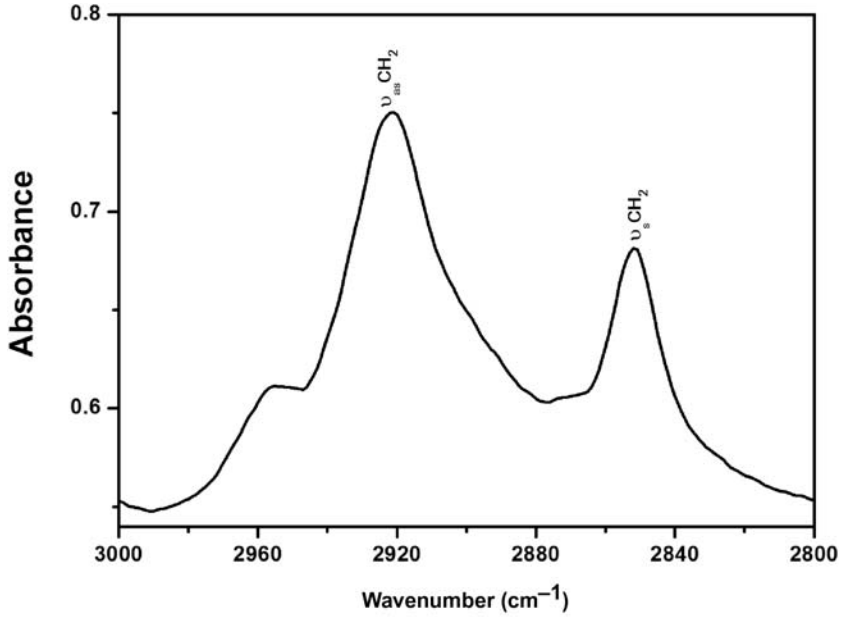


FIGURE 10.2 Fourier transform infrared spectra in the 3000- to 2800-cm^{-1} region from a frankfurter (elaborated with pork meat). These spectra were measured from a small portion of samples using CaF_2 window for transmission infrared spectroscopy and spectral influence of proteins and water should be appropriately substracted.

complex foods such as meat products which contain lipids, protein, and water, any spectral influence of proteins and water should be appropriately subtracted (Herrero et al., 2012) before analyzing lipid structural features in this spectral region.

Additionally, the strong band of the C = O stretching vibration of the carbonyl groups of triglycerides is observed at about 1745 cm^{-1} (Ayora-Cañada et al., 2010). Some bands appearing in the 1400- to 1200-cm^{-1} region can be attributed to bending vibrations of CH_2 and CH_3 aliphatic groups, and the 1125- to 1095-cm^{-1} region includes characteristic bands of C−O the stretching vibration ester groups. Finally, below 1000 cm^{-1}, the band near 723 cm^{-1} is due to the overlapping of the CH_2 rocking vibration and the out-of-plane vibration of *cis*-di-substituted olefins. The major changes occurring in this IR band are due to oxidation (van der Voort et al., 1994). The 723-cm^{-1} band decreases in accordance with the loss of *cis* double bonds due to their isomerization to *trans* groups and/or their breakdown to produce secondary oxidation products. A small absortion increase can generally be observed in the 1400- to 1000-cm^{-1} region. The absorbance increasing in the 1735- to 1660-cm^{-1} region due to oxidation is attributed to carbonyl compounds formed during oxidation. Also, a significant decrease in intensity

is observed for the band located at 3008 cm^{-1}, which derives from the loss in *cis* double bonds (Ayora-Cañada et al., 2010).

Water

A broad absorption band in the 3600- to 3100-cm^{-1} region corresponds to the characteristic O−H stretching vibration and hydrogen bond of the hydroxyl groups. It has been well established that there are two competing structures in the short living molecular order in water: a tetrahedral ice-like arrangement and a nontetrahedral arrangement (McDonald et al., 1986; Marechal, 1991; Kusualik et al., 1994). The component bands of the O−H stretching spectral profile of water in the 3650- to 3000-cm^{-1} region are broadly assigned to two categories. The bands at lower frequencies, approximately 3300- to 3000-cm^{-1}, correspond to the strong hydrogen-bonded patches of molecules with a tetrahedral structure whereas the bands at higher frequencies (3650- to 3300-cm^{-1}) are related to the weak hydrogen-bonded water molecules. Several studies have also shown that water molecule dynamics are significantly disturbed in confined geometries owing to interaction with hydrophilic or hydrophobic substrates at various levels of hydration (Benham et al., 1989; Bergman and Swenson, 2000; Brovchenko et al., 2000; Zanotti et al., 1999).

10.2.3.2 Raman Spectrum

Proteins

Raman spectroscopy provides information on the peptide backbone structure, mainly by means of the amide I and III bands, which are directly related to secondary structure, and the effect of peptide structure on the environment of some side chains such as those of aliphatic, tyrosine, and tryptophan residues, and on the local conformations of disulfide bonds and methionine residues related to tertiary protein structure (Tu, 1982; Tuma, 2005). The characteristic protein Raman bands of interest are described below.

Amide I and III bands: The most useful Raman bands for determining the secondary structure of meat protein (α-helix, β-sheet, turn, unordered) correspond to the amide I and amide III vibrational modes. Fig. 10.3 shows a typical FT-Raman spectrum of a low-fat frankfurter in the 600- to 1800-cm^{-1} region showing both amide I and amide III bands. The contribution of lipids and water molecules in the Raman spectra have to first be appropriately subtracted from the spectra to study protein Raman bands (Alix et al., 1988; Herrero et al., 2008b; Herrero et al., 2014). The strong band centered around 1650- to 1658-cm^{-1} has been assigned unambiguously to the amide I vibrational mode (Krimm and Bandekar, 1986), which involves mainly C = O stretching. Generally speaking, proteins with high α-helical content show an amide I band centered around 1650- to 1658-cm^{-1} (Fig. 10.3), while those with predominantly β-sheet structures show the band between 1665 and 1680 cm^{-1}, and a high proportion of unordered structure is attributable to

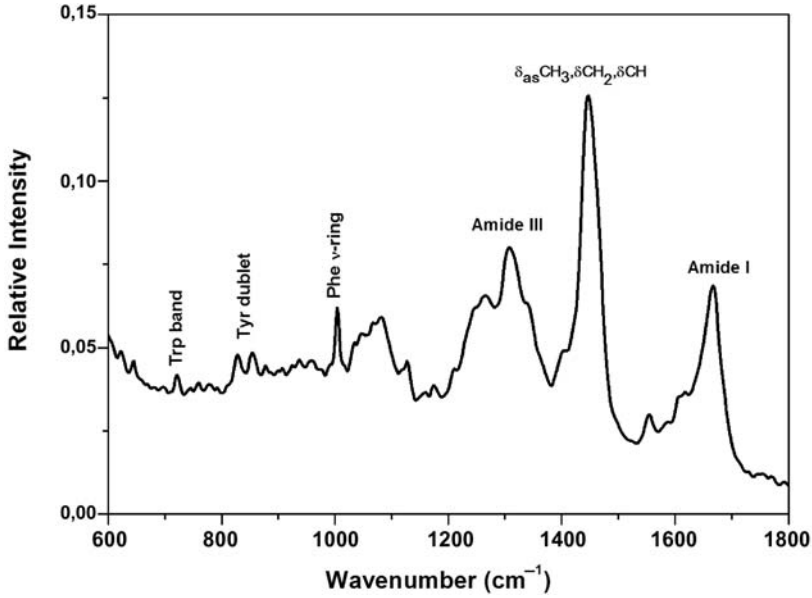


FIGURE 10.3 Typical Fourier transform-Raman spectrum of a low-fat frankfurter (elaborated with pork meat) in the 600- to 1800-cm^{-1} region, where the contribution of lipids and water molecules have been appropriately subtracted from the spectra.

proteins with an amide I band centered at 1660−1665 cm^{-1}. The spectral profile of the amide I band is used to quantify the secondary structure of proteins in terms of α-helix, β-sheet, turn, and unordered (Williams 1983; Alix et al., 1988). However, the contribution of O−H bending vibrations of water molecules in the 1640-cm^{-1} region of Raman spectra has to first be subtracted from the spectra (Alix et al., 1988).

The so-called amide III modes involve C−N stretching and N−H in-plane bending vibrations of the peptide bond as well as contributions from Cα-C stretching and Ci=O in-plane bending. The amide III band is widely used to confirm the results obtained from the amide I band, but is difficult to interpret because proteins produce a complex pattern of bands in the 1225- to 1350-cm^{-1} range (Fig. 10.3) (Krimm and Bandekar, 1986; Pelton and McLean, 2000; Schweitzer-Stenner, 2006).

C−C stretching bands: C−C stretching vibrations in the 890- to 1060-cm^{-1} range are characteristic of α-helix (890−945 cm^{-1}) and β-sheet (1020−1060 cm^{-1}) structures. The gradual loss of these structures has been shown to lead to broadening and weakening in the intensity of this band (Tu, 1982).

Tryptophan residues bands: Tryptophan (Trp) vibrations are visible at 544, 577, 760, 879, 1014, 1340, 1363, 1553, and 1582 cm^{-1} (Thomas, 2002; Combs et al., 2005). A high-intensity ratio of I_{1360}/I_{1340} indicates a

hydrophobic environment, whereas a low-intensity ratio indicates that the Trp residue is involved in the H-bonding of a hydrophilic environment. Modifications in the tertiary structure of proteins can be accompanied by exposure of buried tryptophan residues in proteins, which is observed by a decrease in intensity at 760 cm^{-1} (Tu, 1986) (Fig. 10.3).

Tyrosyl doublet: The ratio of the tyrosyl (Tyr) doublet at 850 and 830 cm^{-1} (Fig. 10.3) is known to be a good indicator of the hydrogen bonding of the phenolic hydroxyl group. The Tyr doublet ratio has been proposed for determining whether the Tyr residue is solvent exposed or buried. When the intensity of the 850-cm^{-1} band is higher than that of the band near 830 cm^{-1} the Tyr residue is exposed, involving changes in the tertiary structure, whereas $I_{850} < I_{830}$ could be interpreted as indicating an increase in the extent to which the tyrosine residues are buried within the protein network (Tu, 1986; Thomas, 2002).

Aliphatic amino acids bands: The bands assigned to the CH_2 scissoring and CH_3 antisymmetric bending vibrations ($\delta_{as}CH_3$, δCH_2, δCH) is observed near 1450 cm^{-1} (Fig. 10.3), and a decrease in the relative intensity of this band may result from hydrophobic interactions of aliphatic residues (Verma and Wallach, 1977).

The bands near 2860, 2935, and 2970 cm^{-1} are assigned to C−H stretching motions ($\nu_s CH_2$ (2860 cm^{-1}), $\nu_{as}CH_2$ (2935 cm^{-1}), and $\nu_{as}CH_3$ (2970 cm^{-1})). It has been shown that exposure of the aliphatic hydrophobic side chains of proteins to an aqueous environment results in increasing intensity of these bands (Verma and Wallach, 1977).

Other bands in the Raman spectra of proteins: A band at 1003−1006 cm^{-1} is attributed to the ν_{12} in-plane ring deformation of the phenylalanine (Phe) ring (Fig. 10.3). The intensity and location of the phenylalanine band is reported to be insensitive to conformation or microenvironment and therefore it may be used as an internal standard (Tu, 1986).

Proteins and peptides with disulfide bonds of cystine show a band centered at 510 cm^{-1} when the C−C−S−S−C−C group is in a "*gauche-gauche-gauche*" conformation. For disulfide bonds in the "*gauche-gauche-trans*" and "*trans-gauche-trans*" conformations, additional bands appear at 525 and 540 cm^{-1}, respectively (Li-Chan et al., 1994). The C−S bonds of methionine and cysteine residues show their stretching vibrations in the 600- to 750-cm^{-1} region, depending on the conformation of the C−S bond. For example, the C−S bond of methionine in the *trans* form shows stretching bands at 655 and 724 cm^{-1}, whereas in the *gauche* form a band appears at around 700 cm^{-1} (Li-Chan et al., 1994). The bands generated by disulfide and C−S bonds in most cases are masked by the librational broad band of water and so often cannot be observed.

A weak Raman band in the 2550- to 2580-cm^{-1} region is attributed to stretching vibration of the S-H group of cysteine residues (Li-Chan et al., 1994).

Aspartic and glutamic acid residues show a Raman band in the 1400- to 1420-cm^{-1} region attributed to the symmetric O−C−O stretching mode of carboxylate groups. The C=O stretch of nonionized COOH groups exhibits a band in the 1700- to 1720-cm^{-1} range. The relative intensity of these bands has been proposed for determining the ionization state of the carboxyl groups (Tu, 1986).

In D_2O solutions of histidine and proteins containing histidine residues, a strong band appears near 1410 cm^{-1}, which can be used to monitor the ionization state of the imidazolium functional group of this amino acid (Tu, 1986).

Proline and its derivative hydroxyproline, which is commonly found in collagen, show strong Raman scattering due to the pyrrolidine ring. Two bands for proline and another for hydroxyproline residues appear at 921 and 855 cm^{-1}, and 880 cm^{-1}, respectively (Herrero, 2008b).

A Raman band at 160 cm^{-1} is attributed to restricted translational motions of water molecules involved in hydrogen bond interactions. The shifting of the band maximum toward lower frequencies has been explained in terms of water molecules binding to biomolecules such as proteins (Colaianni and Nielsen, 1995; Gniadecka et al., 1998).

Lipids

Raman bands observed in the spectral region below 1800 cm^{-1} can be found at about 1750, 1660, 1440, and 1295 cm^{-1}, and are assigned undoubtedly to the C=O stretching modes, C=C stretching modes, CH_2 scissoring modes, and CH_2 twisting modes of lipids, respectively (Li-Chan et al., 1994; Baeten et al., 1996).

Raman spectroscopy can provide a fast quantitative analysis of the unsaturation degree, *cis*/*trans* isomers ratio as well as the amount of double bonds in lipid hydrocarbon chains (Li-Chan et al., 1994; Baeten et al., 1996). The unsaturation degree of lipids has been measured either by means of area ratio of the C=C stretching band located between 1600 and 1700 cm^{-1} and the C=O stretching band falling in the 1790- to 1710-cm^{-1} range, or by means of area ratio of the C=O stretching band and the scissoring band of CH_2 groups (1543−1382 cm^{-1}). The *cis*/*trans* isomers ratio has been quantified also by considering relative intensities of the 1657- and 1667-cm^{-1} bands, respectively. In addition, the total content of *cis* isomer has been measured from the $I_{1265\ cm^{-1}}/I_{1303\ cm^{-1}}$ intensity ratio, whereby results have been obtained which are close to those provided by gas chromatography analysis (Larsson and Rand, 1973). These studies showed also that the changes in the environment of lipid hydrocarbon chains due to different phases resulted in great differences in the C−H stretching region of Raman spectrum.

Although Raman spectroscopy has not been often used for monitoring edible oil oxidation, it has been reported that the major changes during

oxidation comprise intensity decreasing of a broad band at about 1680−1720 cm^{-1} due to the development of carbonyl compounds (Muik et al., 2005). The 1725-cm^{-1} band is assigned to the C=O stretching of saturated aldehydes and that around 1690 cm^{-1} is assigned to the C = O stretching of conjugated unsaturated aldehydes.

The region between 2800 and 3050 cm^{-1} (Fig. 10.4) is characteristic of the symmetric and asymmetric CH stretching vibrations of methyl and methylene groups of lipids, and can be analyzed once the influence of water molecules and proteins in this Raman spectral region has been properly eliminated (Alix et al., 1988; Herrero et al., 2014). In this region, there are various prominent bands: a CH_3 symmetric stretching band near 2897 cm^{-1}, a CH_2 asymmetric stretching band near 2930 cm^{-1} and a CH_2 symmetric stretching motion near 2850 cm^{-1} (Fig. 10.4). Another Raman band can be found at about 3007 cm^{-1} (Fig. 10.4) attributable to *cis*-olefinic group =C-H stretching vibration (Muik et al., 2005; Zou et al., 2009). The symmetric and asymmetric vibrational modes of CH_2 and CH_3 groups can provide insights into interactions between hydrocarbon chains in various states, either in polymorphic forms, emulsions, or in lipid−protein interactions. It has been reported that the peak height intensity ratios $I\nu_sCH_2/I\nu_{as}CH_2$ (I_{2850}/I_{2890}) and $I\nu_sCH_3/I\nu_{as}CH_3$ (I_{2935}/I_{2890}) provide useful indices for gauging lipid packing effects, and determining relative order/disorder of the intermolecular lipid chain (Larsson and Rand, 1973; Larsson, 1976; Carmona et al., 1987; Levin and Lewis, 1990). In particular, the $I\nu_sCH_2/I\nu_{as}CH_2$ index reflects

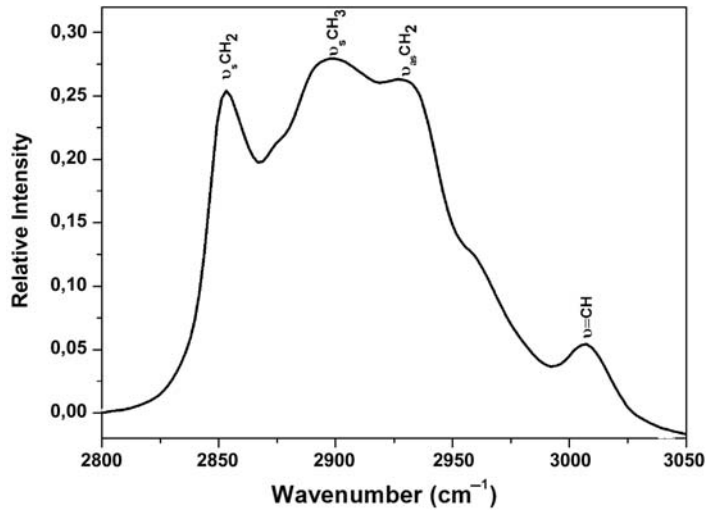


FIGURE 10.4 Raman spectra in the 2800- to 3050-cm^{-1} region from a frankfurter (elaborated with pork meat) where the contributions of lipids and water molecules have been appropriately subtracted from the spectra.

primarily interchain interactions, whereas the $I\nu_{as}CH_2/I\nu_sCH_3$ intensity ratio measures effects originating from changes in intrachain *trans*/*gauche* isomerization superimposed on the chain−chain interactions (Levin and Lewis, 1990).

Water Molecules

The structure of water is reflected in the Raman spectrum through a band appearing near 180 cm^{-1}. This band constitutes direct evidence of hydrogen bonding, and its intensity has been explained as dependent on the concentration of O−H•••O units (Walrafen and Fisher, 1986; Maeda and Kitano, 1995). The band of free water at 180 cm^{-1} shifts toward lower frequencies (120−160 cm^{-1}) when the H_2O molecular species are attached to macromolecules (bound water). This band is attributable to vibrational modes of atoms involved in hydrogen bonding occurring, for instance, in water−protein and protein−protein interactions. The intensity decreasing or absence of the 180-cm^{-1} band is indicative of loss of hydrogen bonds.

The intramolecular vibrations of hydrogen-bonded water molecules comprise two OH stretching bands in the 2800- to 3500-cm^{-1} region and one band of angular deformation appearing around 1645 cm^{-1} (Maeda and Kitano, 1995). This deformation band is characterized by low intensity, and hence is masked by the amide I band located near 1650 cm^{-1}. Some authors have established certain correlations between the microscopic sizes of water domains and the spectral profile of the OH band in the 3000- to 3500-cm^{-1} region (Lafleur et al., 1989).

10.3 APPLICATION OF VIBRATIONAL SPECTROSCOPY FOR MEAT QUALITY ASSESSMENT

Vibrational spectroscopy combined with chemometric analysis has been gaining popularity as a tool to assess meat quality, since there seems to be a significant relationship between structural changes determined through spectral data and sensorial and technological properties results evaluated with methodologies commonly used to determine quality in meat. Chemometric methods in spectroscopic analysis are mathematical or statistical methods used to handle the spectroscopic data. In this context, different multivariate statistic methods are applied to the spectroscopic data to assess meat quality such as: principal component analysis (PCA), partial least-squares regression (PLSR), multiple linear regressions (MLR), classical least-square (CLS), artificial neural network, and many other methods. The advantage of multivariate statistics is that they permit analysis of numerous spectra and spectral regions simultaneously. Additionally, vibrational spectroscopy has considerable advantages that are relevant when searching for a tool for quality assessment, namely high specificity, no need for sample preparation, noninvasive,

need for only a small amount of sample, and it provides information about different compounds at the same time, among others. Another advantage is the fact that over recent years, handheld instruments have been developed that have led to a further extension of the range of applications of vibrational spectroscopy for the quality assessment of food. In particular, Raman and IR portable handheld devices have been developed to predict meat quality and demonstrate their potential (Zamora-Rojas et al., 2012; Roza-Delgado et al., 2014; Scheier et al., 2015; Fowler et al., 2015).

Numerous studies exist related to the potential of vibrational spectroscopy to determine meat composition, particularly fat content and fatty acid composition (Ripoche and Guillard, 2001; Damez and Clerjon, 2013). However, the relevant application of these spectroscopic techniques to asses meat quality should be noted, as compared with conventional methods used with this aim such as sensory analysis, physicochemical and microbiological methods.

10.3.1 Vibrational Spectroscopy and Sensory Analysis

NIR spectroscopy has been utilized as a technique to quickly evaluate some sensorial properties of meat such as appearance (color, marbling, etc.), odor, flavor, juiciness, tenderness, or firmness (Park et al., 1998; Venel et al., 2001; Liu et al., 2003; Andres et al., 2007; Prieto et al., 2009). The potential of visible and near-IR reflectance NIR spectroscopy to predict sensory characteristics related to the eating-quality of lamb meat samples has been evaluated (Andres et al., 2007). Results evidenced that the most important regions of the spectra to estimate the sensory characteristics are those related to the absorbance of intramuscular fat and water in meat samples. However, NIR spectra present often overlapped to yield broad bands that do not provide high-resolution spectroscopic fingerprints of different molecular functional groups, which subsequently limit the accuracy of the sensorial profiling of the meat. Analysis of a meat sample using an mid-infrared (MIR) spectrum ($4000-400\ cm^{-1}$) probably gives more accurate data. In this context, the potentials of both NIR and FT-IR spectroscopy have been evaluated to investigate meat aging and salting with different types of salt in order to relate spectral results directly to water-binding properties and sensorial attributes (Perisic et al., 2013). HSI techniques based on NIR have received much attention for prediction of the tenderness of meat (Cluff et al., 2008; Naganathan et al., 2008; ElMasry et al., 2012b). Kamruzzaman et al. (2013a,b) developed and tested a HSI system (900−1700 nm) to predict sensory tenderness of lamb meat obtaining partial least squares regression (PLSR) models with reasonable accuracy. The feasibility of predicting sensory characteristics of chicken breasts deboned at 2, 4, 6, and 24 h postmortem by visible/NIRS in the 400- to 1850-nm region was also determined (Liu et al., 2004).

Raman spectroscopy is an alternative to IR spectroscopy to predict some sensorial attributes of meat and meat products (Brøndum et al., 2000; Beattie

et al., 2004; Wang et al., 2012). In these respect, Raman spectra data have been correlated with sensory attributes (juiciness and texture) using PLSR from cooked beef samples (Beattie et al., 2004). A strong positive correlation between protein characteristics Raman bands (amide I band, C−C stretching bands, and aliphatic amino acids bands) and juiciness determined by sensory analysis were found. These results could be related to changes in secondary structure, in terms of increase of β-sheets and decrease of α-helices, as wells as to hydrophobic interactions of aliphatic residues that can be associated to juiciness. Raman data also showed good correlation between with amide I and amide III bands (characteristics of proteins) and textural sensory attributes such as toughness. This fact has been attributed to the increase in β-sheets content in the tough meat. Other Raman bands assigned to protein such as tryptophan residues bands and tyrosyl doublet were related to tough meat samples (Beattie et al., 2004). Some authors reported that some sensory attributes of pork loins are moderately correlated to Raman spectroscopic data in the 400- to 2000-cm^{-1} region (Wang et al., 2012). They developed PLSR models to predict the value of sensory tenderness, chewiness, and juiciness based on Raman spectroscopic data of pork loins. Furthermore, these authors created binary barcodes models based on spectroscopic data to classify pork loins into sensory quality grades ("good" and "bad" in terms of tenderness and chewiness).

10.3.2 Vibrational Spectroscopy and Physicochemical Methods

IR and Raman spectroscopy have been compared to different traditional physicochemical methods (water-holding capacity (WHC), instrumental textural methods, etc.) to asses meat quality treated under different conditions of handling, processing, and storage, mainly through the changes in proteins, water, and lipids in the spectra.

The WHC of meat, defined as the ability to hold its own and/or added water, is one of the most important technological properties of meat because it affects the quality of the end product and can influence consumer preferences (Honikel and Hamm, 1994). Water loss is undesirable in meat due to its influence on the appearance, juiciness, and cooking yield, etc. IR spectroscopy and mainly NIR have been used to evaluate the WHC of meat and meat products such as broiler breast fillets (Hoving-Bolink et al., 2005; Prieto et al., 2009; Samuel et al., 2011; Bowker et al., 2014). The results have shown that there is much controversy in relation to the possibilities of NIR as a potential technique to predict WHC in meat, although some authors reported moderate predictions for this technological property (Prieto et al., 2009; Ripoll et al., 2008; Forrest et al., 2000; Bowker et al., 2014; Barbin et al., 2015). NIR revealed WHC associations with protein, intramuscular fat, and water. Some authors indicated that the most important wavelengths identified for build prediction models of WHC in chicken were 440, 558,

1656, and 1908 nm (Barbin et al., 2015). Linear discriminate analyses were used to classify fillets as high- or low-WHC according to predicted meat quality characteristics. Fillets could be correctly classified into high- and low-WHC groups using the visible spectra (400−750 nm) with 88%−92% accuracy and using the NIR spectra (750−2500 nm) with 74%−76% accuracy (Bowker et al., 2014). Additionally, a HSI technique in VIS/NIR region was tested to investigate the ability of the technique for determining WHC in red meat accompanied with multivariate analysis by identifying dominant feature wavelengths related to WHC (ElMasry, Sun and Allen, 2011; Kamruzzaman et al., 2016a). Studies in fresh pork meat revealed a high correlation between WHC and FT-IR spectra using PLSR. Pedersen et al. (2003) indicated that the IR region 1800−900 cm^{-1} contains the best predictive information which covers the functional group frequencies of water, protein, fat, and glycogen, including the carbonyl and amide groups. This research included the possibilities of Raman spectroscopy to determine WHC and showed that Raman spectral regions of interest to WHC according to PLSR results are related to protein conformation (Pedersen et al., 2003). In particular, WHC has been related to changes in Raman region containing NH stretching bands of amide groups in proteins (3071–3128 cm^{-1}) and C−C stretching bands (876−951 cm^{-1}) which indicated modifications in secondary structure of proteins (α-helix structure) (Pedersen et al., 2003). Raman spectroscopy also provided good predictive information for WHC of chicken breast meat using specific bands (538, 691, 1367, 1743 cm^{-1}) and amide I and III regions (Phongpa-Ngan et al., 2014).

Other technological properties which define in many cases the quality of muscle-based foods, since they determine to a great extent the product acceptance by consumers, are textural characteristics. Textural characteristics are associated with the intrinsic structure and properties of components of the muscle. Myofibrillar proteins, among the meat muscle components, are the main contributors imparting textural attributes. There are several instrumental methods to determine textural properties of meat, among them texture profile analysis (TPA), the Kramer shear-compression cell method, the puncture test, the Warner−Bratzler cell method, etc. (Bourne, 2002). In this context, Kamruzzaman et al. (2013a,b) developed and tested a HSI system (900−1700 nm) to predict instrumental tenderness of lamb meat. The Warner−Bratzler cell method was performed and shear force (WBSF) values were used as instrumental texture parameters. They obtained PLSR models with reasonable accuracy. The results confirmed that the spectral data could become an interesting screening tool to quickly categorize lamb steaks into good (i.e., tender) and bad (i.e., tough) based on WBSF values and sensory scores (Kamruzzaman et al., 2013a,b). Since WBSF is an important index of meat tenderness the possibilities of Fourier transform near-IR (FT-NIR) were also evaluated to determine this texture parameter as well as others (Andrés et al., 2008; Bowling et al.,

2009; Cai et al., 2011). Some authors performed a synergy interval partial least square (SI-PLS) algorithm to calibrate a regression model for WBSF. Experimental results showed that the correlations coefficients in the calibration set (R_c) and prediction set (R_p) were 0.7533 and 0.7041, respectively, for the WBSF model. The overall results demonstrated that NIR spectroscopy combined with SI-PLS could be utilized to determine WBSF in pork (Cai et al., 2011). The feasibility of predicting shear force of chicken breasts deboned at 2, 4, 6, and 24 h postmortem by visible/NIRS in the 400- to 1850-nm region was determined (Liu et al., 2004). On the basis of predicted shear values from the partial least-squares (PLS) model, breast samples were classified into "tender" or "tough" classes with a correct classification of 74.0%. FT-IR and FT-Raman spectroscopy were useful to relate textural behavior and structural characteristics in meat and meat products (Herrero et al., 2008c; Xu et al., 2011; Carmona et al., 2011; Herrero et al., 2012, 2014). These spectroscopic techniques have been used to evidence structural changes in meat components during processing and storage that have been correlated with textural properties of the product. In particular, these techniques have been used to study the relationship between texture of meat products and conformational modifications in meat components induced by storage and processing such as different salt concentrations, heating, or the incorporation of nonmeat ingredients (soy protein, kappa-carrageenan, wheat dietary fiber, cold gelling agents, olive oil bulking agents, etc.), in most cases with the aim of developing healthy foods (Chattong et al., 2007; Herrero et al., 2008c, d, 2012, 2014; Carmona et al., 2011; Shao et al., 2011; 2015; Gao et al., 2015). In general the incorporation of these ingredients produced frequency upshifting and profile modifications of amide I attributed to an increase of β-sheet structure. In most studies, structural changes in proteins and lipids have been seen to be correlated with specific textural characteristics of meat products (Shao et al., 2011; Herrero et al., 2014, Gao et al., 2015).

Both IR and Raman spectroscopy have also been used to predict other meat physicochemical quality attributes commonly used as a quality index for the meat industry and meat science research such as color, pH, markers of deterioration, etc. (Prieto et al., 2009; Cai et al., 2011; Barbin et al., 2015; Fowler et al., 2015). However, since some cases such as studies evaluating NIR spectroscopy to predict pH or color (in terms of lightness (L^*), redness (a^*) and yellowness (b^*) parameters) the results obtained are contradictory (Prieto et al., 2009), a NIR HSI system has been also used for predicting pH and L* values in beef, lamb, and pork (ElMasry et al., 2012b; Kamruzzaman et al., 2012; Barbin et al., 2012). Recently, a visible NIR HSI system in the spectral range of 400−1000 nm was tested to develop an online monitoring system for red meat (beef, lamb, and pork) color (L^*, a^*, b) in the meat industry (Kamruzzaman et al., 2016b). Only

six wavelengths (450, 460, 600, 620, 820, and 980 nm) were further chosen as predictive feature wavelengths for predicting L^*, a^*, and b^* in red meat. Multiple linear regression models were then developed and predicted L^*, a^*, and b^* with coefficients of determination (R) of 0.97, 0.84, and 0.82, respectively. The results indicated that HSI has the potential to be used for rapid assessment of meat color. Some studies were conducted to determine the potential for a Raman spectroscopic handheld device to predict meat quality traits of fresh lamb (Fowler et al., 2015; Scheier et al., 2015). Results indicated a potential to predict pH and L^* values among other parameters.

10.3.3 Vibrational Spectroscopy and Microbiological Analysis

Microbial spoilage plays a very important role in food safety and quality evaluation. The degree of microbiological spoilage which the meat has undergone affects product safety and quality. Muscle food is one of the most perishable food products because of its vulnerability to microbial spoilage, which can result in critical food safety problems. Traditional techniques for detection and evaluation of microbial spoilage in muscle foods have several drawbacks. They are tedious, laborious, destructive, and time-consuming. Some rapid microbiological methods have been developed during the last decade and some of these automated procedures could be used. However, in the majority of rapid methods, large numbers of samples are needed. Therefore, it is recommended that novel techniques be developed that limited these drawbacks. In this respect, vibrational spectroscopic techniques could be a promising alternative. Recently, several studies have shown that the HSI system in the visible and near-IR range (400−1100 nm), in combination with other classical microbiological methods and chemometric analysis as PLSR models, was able to detect the bacterial spoilage in chicken meat (Feng and Sun, 2012; Xiong et al., 2015; Cheng and Sun, 2015). Results demonstrated the feasibility of using this spectroscopic technique as a valid means for nondestructive determination of total viable counts (TVC) in chicken fillet samples, as well as detecting specific bacteria such as *Enterobacteriaceae* and *Pseudomonas* using selected wavelengths (Feng and Sun, 2013; Feng et al., 2013). Some authors have also evaluated the possibilities of FT-IR to be used for the rapid assessment of meat spoilage (TVC, *Pseudomonas* spp., lactic acid bacteria (LAB), *Enterobacteriaceae*, etc.) (Ellis et al., 2002; 2004; Ammor et al., 2009; Papadopoulou et al., 2011; Argyri et al., 2013; Cheng and Sun 2015). FT-IR was used directly from the sample surface using ATR, while in parallel the TVC of bacteria were obtained by traditional microbiological methods (Ellis et al., 2004; Ammor et al., 2009). Quantitative interpretation of FT-IR spectra was undertaken using PLSR and allowed for accurate estimates of bacterial loads to be calculated directly from the meat surface in 60 s (Ellis et al., 2004).

Machine-learning methods in the form of genetic algorithms and genetic programing were used to elucidate the wavenumbers of interest related to the spoilage process. The results obtained demonstrated that, using FT-IR and machine learning, it was possible to detect bacterial spoilage rapidly in beef and that the most significant functional groups selected could be directly correlated to the spoilage process which arose from proteolysis, resulting in changes in the levels of amides and amines (Ellis et al., 2004). FT-IR spectra of poultry meat containing specific bacteria (*Salmonella enteritidis*, *Pseudomonas ludensis*, *Listeria monocytogenes* and *Esherichia coli*) were collected and investigated for their classification and quantification (Grewal et al., 2015). Multivariate data analysis techniques PCA, PLS discriminant analysis (PLSDA) and soft independent modeling of class analogy (SIMCA) were used and the highest correct classification results for SIMCA and PLSDA were achieved in the 1200- to 1800-cm^{-1} spectral region. Thus it is seen that FTIR spectroscopy in combination with chemometrics is a powerful technique that can be further developed to differentiate between bacteria on poultry meat surface (Grewal et al., 2015).

Raman spectroscopy coupled with chemometric models is gaining increasing attention for rapid and precise detection of microorganisms (Lu et al., 2011; Cheng and Sun, 2015; He and Sun, 2015). In particular in meat, a combination of Raman microspectroscopy and multivariate analysis (cluster analysis, support vector machine, etc.) was applied to detect pathogens in meat (Meisel et al., 2014). *L. monocytogenes, Salmonella spp.,* ***Staphylococcus*** *aureus and* ***Yersinia*** *enterocolitica* were identified after isolation from artificially contaminated minced beef or chicken breast by a three-level classification model based on support vector machines. Models predictive of the microbiological load were calculated using chemometric analysis (PLSR, genetic programing (GP), genetic algorithm (GA), artificial neural networks, etc.) and Raman spectra data from minced beef samples stored under different packaging conditions (aerobic and modified atmosphere packaging) at 5°C (Argyri et al., 2013). In general, it was observed that for calibration models, better predictions were obtained for TVC, LAB and *Enterobacteriaceae*. The bands that were mostly used were from 2096 to 2140 cm^{-1} and from 3296 to 3400 cm^{-1} for all cases.

In addition, HSI systems have shown their potential for inspecting fecal contaminants in real-time mode during poultry and broiler processing (Windham et al., 2005; Park et al., 2011). The line-scan imaging system utilized an electron-multiplying charge-coupled device (EMCCD) camera and an imaging spectrograph (400−1000, Hyperspect-VNIR) attached to the EMCCD camera (Park et al., 2011). The authors concluded that more research should be carried out to fully validate the performance of this system to detect fecal contaminants for high detection accuracy with minimum false-positive errors.

10.3.4 Vibrational Spectroscopy and Authentication

Meat authenticity is an emerging area of concern for consumers, retailers, and food regulatory bodies, as it guarantees that food has not been subjected to adulteration by any lower-grade material, either by accident of for economic gain. Moreover, meat adulteration can cause some religious problems as, in some countries, with the consumption of certain proscribed meats (e.g., pork). In this concern, vibrational spectroscopic methods are emerging as potential tools for the detection of meat adulteration (Al-Jowder et al., 1999; Reid, O'Donnell and Downey, 2006; Herrero, 2008a; Kamruzzaman et al., 2013a,b; Qu et al., 2015). NIR spectroscopy has been shown to be a potential tool for discrimination of beef from other types of meat, as well as differentiation between different types of beef meat involving various breeds, muscle types, and ages as well (Qu et al., 2015). Preliminary studies has been performed to develop and optimize a rapid analytical technique based on NIR HSI to detect the level of adulteration in minced lamb (Kamruzzaman et al., 2013a,b). Initial investigations were carried out using PCA to identify the most potential adulterate in minced lamb (such as minced pork) and the PLSR model to predict their level of adulteration. The results demonstrated that the laborious and time-consuming tradition of analytical techniques could be replaced by spectral data in order to provide a rapid, low-cost, and nondestructive testing technique for adulterate detection in minced lamb meat (Kamruzzaman et al., 2013a,b). Additionally, it has been reported that mid-IR spectroscopy with attenuated total reflectance (FT-IR-ATR) in conjunction with appropriate chemometrics methods have the potential to be established as a technique for meat authentication (Al-Jowder et al., 1997; 1999; 2002). FT-IR-ATR, PCA, and PLSR have shown their possibilities to distinguish minced chicken, pork and turkey meat from their IR spectra (Al-Jowder et al., 1997). This spectroscopic technique can also discriminate between beef muscle and offal tissue types using PLSR and canonical variate analysis (Al-Jowder et al., 1999).

Some investigations have been performed in meat to evaluate the possibilities of Raman spectroscopy for authentication of meat by substitution of traditional techniques such as detection of DNA or RNA, immunological, electrophoretic, and chromatographic techniques which are destructive and time consuming. In this respect, Raman spectroscopy has been used to discriminate between main meat-type poultry species (turkey and chicken) and anatomical muscle origin (breast and leg) (Ellis et al., 2005). Cluster analysis using Raman spectra in the 0- to 3000-cm^{-1} range showed that the major discrimination ability was between leg and breast meat (Ellis et al., 2005). Other studies have focused on beef offal (i.e., kidney, liver, heart, and lung) adulteration of beef burgers using dispersive Raman spectroscopy and multivariate data analysis to explore the potential of these analytical tools for detection of adulterations in comminuted meat products with complex formulations (Zhao

et al., 2015). Raman spectral data in the fingerprint range (900−1800 cm^{-1}) were examined using both a classification (PLS-DA) and class-modeling (SIMCA) approach to identify offal-adulterated and authentic beef burgers. PLS-DA models correctly classified 89%−100% of authentic and 90%−100% of adulterated samples. Additionally, a novel fat-based method was developed which uses Raman spectroscopy in combination with PCA for the rapid determination of beef adulteration with horsemeat (Boyaci et al., 2014a, b). In order to distinguish pure horse and beef samples from each other, PCA was performed on the entire spectrum between 200 and 2000 cm^{-1} and results showed that the presence of different concentrations (25, 50, and 75%, w/w) of horsemeat in beef was differentiated (Boyaci et al., 2014a).

10.4 CONCLUSIONS

The combination of methods based on vibrational spectroscopy (IR and Raman spectroscopy) with multivariate statistical techniques (chemometrics) has considerable potential and a growing range of uses in the food field, particularly in applications for meat quality assessment. IR spectroscopy has been, and will continue to be, an important analytical tool for this purpose. Even though IR analyzers have already taken part in the process development, scale-up and on the floor of production facilities, the evolution of the technology will continue to bring newer IR instruments that are more flexible, rugged, robust, and easier to implement and operate. Raman spectroscopy is also being used in this area since rich spectral information, sharp well-resolved spectral bands, relative ease of sampling, and process interfacing, provide advantages over many traditional analysis tools. Raman spectroscopy can often reliably predict qualitative and quantitative aspects of meat components and, as Raman technology continues to develop, a wider range of tools and applications in this field will become available. Additionally both IR and Raman spectroscopy show some advantages when compared with traditionally used methodology, since this technique involves analytical direct methods (noninvasive), needs few sample milligrams, and provides information about different meat components at the same time in a unique spectra.

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Part III

The Current Qualities of Consumer and Public Perceptions; What Is Sustainable, Ethical, Desirable, and Healthy

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Chapter 11

Poultry Meat Nutritive Value and Human Health

Alessandra Bordoni and Francesca Danesi
University of Bologna, Bologna, Italy

11.1 INTRODUCTION

When gauging the relationship between nutrition and health, animal-derived foods (and meat in particular) are usually assessed in a global manner: the lay public perception of their effects on wellbeing and health is consequently often quite negative. However, various aspects of this relationship deserve to be analyzed in detail, in particular the relevant differences that characterize the health effects of different meat types. In many cases, the distinction is simply made between red and white meat (Battaglia Richi et al., 2015; Sinha et al., 2009). Recent evidence from large prospective United States and European cohort studies and from meta-analyses of epidemiological studies indicates that the long-term consumption of increasing amounts of red meat and particularly of processed meat is often associated with an increased risk of total mortality, cardiovascular disease, colorectal cancer, and type 2 diabetes, in both men and women. The association is absent with white meat (Battaglia Richi et al., 2015).

Only a few studies have considered the impact of meat on health in relation to the animal species of origin. From a nutritional point of view, the regular consumption of poultry meat has been associated with many positive aspects (Millen et al., 2014). Poultry meat, mainly chicken and turkey, is considered nutritionally more valuable than other meats, and the American Heart Association (http://www.heart.org/) recommendation for the consumption of poultry (chicken and turkey) without the skin are the same as for fish and shellfish, i.e., no more than 6 ounces, cooked, per day.

The term poultry indicates domesticated fowl collectively, especially those valued for their meat and eggs, such as chickens, turkeys, ducks, and geese. Poultry is the second most widely eaten type of meat in the world (FAO, 2014), accounting for about 35% of total meat intake worldwide, compared to pork and beef (36% and 22%, respectively).

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Poultry is available fresh or frozen, as whole birds or as joints (cuts), bone-in or deboned, seasoned in various ways, raw, or ready cooked. The meatiest parts of a bird are the flight muscles on its chest, called "breast" meat, and the walking muscles on the legs, called the "thighs" and "drumsticks." The wings are also eaten (Buffalo wings are a popular example in the United States).

Overall, poultry meat is distinguished for its low energy concentration and its high nutrient density, although many different factors such as the species, the genetic background, the animal's diet, the farming systems (organic, free range, intensive), the cut, the presence of skin, the cooking procedure have been shown to influence some compositional aspects of the meat.

In this chapter, the most important nutrients in poultry meat are described. Their content in poultry meat is reported, eventually considering different cuts of both chicken and turkey meat, and compared to the dietary reference values for healthy nutrition.

11.2 PROTEINS

Poultry meat, as well as other meats, is a good source of high-biological-value proteins (20%–22%).

As evidenced in Table 11.1, protein content is similar in chicken and turkey, and it is poorly influenced by the cut. The increased protein

TABLE 11.1 Protein Content of Some Raw and Cooked (Roasted) Poultry

	Chicken Protein Content (g/100 g)	Turkey Protein Content (g/100 g)
Whole with skin, raw	18.6	21.6
Whole skinless, raw	21.4	22.6
Whole, with skin, roasted	27.3	28.6
Whole, skinless, roasted	28.9	29.1
Breast with skin, raw	20.9	21.9
Breast skinless, raw	22.5	23.3
Breast with skin, roasted	29.8	29.0
Breast skinless, roasted	31.0	29.5
Drumstick with skin, raw	18.1	20.0
Drumstick skinless, raw	19.4	20.5
Drumstick with skin, roasted	23.4	28.2
Drumstick skinless, roasted	24.2	28.6

Data from the USDA National Nutrient Database for Standard Reference, release 28 (2015).

concentration in cooked meat is essentially due to a loss of water during the cooking process (Lofgren, 2005).

In Europe, the Population Reference Intake (PRI), which is equivalent to the amount required to cover the needs of almost all (97.5%) healthy subjects in the population, is estimated to be 0.83 g protein/kg body weight per day for adults of all ages (European Food Safety Authority – EFSA, 2012). Similar values are reported as Dietary Reference Intakes (DRIs) for the American population. It can therefore be estimated that 100 g of raw poultry meat covers about 1/3 of the daily protein requirement for a man weighing 70 kg.

In addition, poultry meat, like other meats, milk, and eggs, has a protein component usually defined as "high quality," and the low content of collagen (a structural protein) increases the digestibility of poultry meat.

11.3 LIPIDS

The total lipid and cholesterol content of some raw and cooked (roasted) poultry is reported in Table 11.2.

Compared to other types of meat, poultry appears to be relatively low in fats, which can increase during preparation due to the addition of butter or oil, and to the water loss during cooking. Lipid and cholesterol content depends on the cut, and mainly on the presence of the skin (which can be easily removed).

Regarding the fatty acid composition of poultry meat, the ratio of saturated fatty acids to unsaturated fatty acids (SFA/UFA) is about 1:3, being lower in breast than in other cuts, and when the skin is removed. An interesting nutritional characteristic of poultry meat is the high content of long-chain n-3 polyunsaturated fatty acids (n-3 LC-PUFA).

The therapeutic and preventive benefits of n-3 LC-PUFA, which occur in fish and fish oil, are well documented (Harris et al., 2013; Salter, 2013). However, consumers that usually do not eat fish are reluctant to modify their dietary habits to follow health claims (IFIC Foundation, 2009). The enrichment of food with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) requires the use of marine sources and consequently seriously affects the pressure on global fish stocks and aquaculture, representing an unsustainable solution in the long term (Naylor et al., 2000). These issues could be solved by providing n-3 fatty acids via the terrestrial food chain. Avian meat (cheaper than fish) represents an important source of n-3 PUFA, and could significantly contribute to the improvement of human nutrition, also taking into account its high consumption rate.

Several studies have demonstrated that it is possible to enrich poultry products (meat and eggs) with n-3 LC-PUFA through dietary strategies (Fraeye et al., 2012; Meluzzi et al., 2001; Rossi et al., 2013), and Boschetti et al. (2016) have highlighted the possibility of a genotype-based selection of strains to produce meat with increased content of n-3 LC-PUFA, due to a higher ability to desaturate/elongate the dietary precursor α-linolenic acid (ALA).

TABLE 11.2 Lipid and Cholesterol Content of Some Raw and Cooked (Roasted) Poultry

	Chicken Lipid Content (g/100 g)	Turkey Lipid Content (g/100 g)	Chicken Cholesterol Content (mg/100 g)	Turkey Cholesterol Content (g/100 g)
Whole with skin, raw	15.1	5.6	75	72
Whole skinless, raw	3.1	1.9	70	67
Whole, with skin, roasted	13.6	7.4	88	109
Whole, skinless, roasted	7.4	3.8	89	101
Breast with skin, raw	9.3	7.5	64	63
Breast skinless, raw	2.6	2.3	73	53
Breast with skin, roasted	7.8	5.3	84	79
Breast skinless, roasted	3.6	2.0	85	70
Drumstick with skin, raw	9.2	6.8	92	82
Drumstick skinless, raw	3.7	4.0	89	79
Drumstick with skin, roasted	10.2	9.4	130	120
Drumstick skinless, roasted	5.7	6.5	130	118

Data from the USDA National Nutrient Database for Standard Reference, release 28 (2015).

11.4 ENERGY

Overall, the energetic value of poultry meats is within the range of other meats, although it varies according to the presence/absence of the skin. In raw meat, the highest value is in chicken thighs (196 kcal/100 g), and the lowest in chicken breast without skin (100 kcal/100 g). In general, the presence of skin increases the caloric value by around 25%−30%. This is due to the high fat content of the skin.

The energetic value of cooked poultry meats is higher, due not only to the possible addition of fats but also to the water loss during the cooking process.

11.5 IRON

Poultry meat is often cited as a healthy food for both general health and dieting, as it is rich in proteins and provides minimal fats. In addition it is a source of a number of minerals, including iron.

Iron is an essential micronutrient. In the human organism, it is part of the structure of many enzymes and proteins, including hemoglobin, which is responsible for carrying oxygen throughout the body. Iron is also involved in the development and differentiation of cells.

Meat represents the main dietary source of highly available iron (heme iron). Accurate knowledge of the levels of both total iron and its chemical forms (heme, nonheme) in meat and diets is of great importance because of the strong difference in availability that heme and nonheme iron display.

Among meats, horse meat has the highest iron content (3.9 mg/100 g), and raw chicken breast has the lowest (0.4 mg/100 g). Percentage of heme iron in total iron in raw chicken breast is 30%. Both total and heme iron markedly differed among the meat cuts (Table 11.3).

Thermal processes that meats undergo have a significant impact on the heme iron content in meat, since heme iron is converted, to varying degrees, into nonheme iron by heat treatments (Igene et al., 1979; Schricker et al., 1982) (Table 11.4). These changes in the heme:nonheme ratio can determine changes in the iron bioavailability from meats or diets. The reduction in heme iron content occurring during cooking can be controlled by using milder processing conditions that promote heme molecule stability.

In adults, the PRI of iron, calculated as the dietary requirement at the 97.5th percentile, is 11 mg/day for men and postmenopausal women. In premenopausal women the PRI is 16 mg/day to cover the requirements of 95% of the population (EFSA, 2015).

Consuming inadequate levels of iron can have serious consequences; one of the most prominent issues is the risk of iron-deficiency anemia. Too little

TABLE 11.3 Total Iron Content and Percentage Heme Iron in Raw Poultry and Other Meats

	Total Iron (mg/100 g)	% Heme Iron
Chicken breast	0.40	30
Chicken thighs	0.70	30
Chicken wings	0.59	38
Turkey breast	0.50	28
Turkey thighs	0.99	50
Beef fillet	2.35	90
Veal fillet	0.85	84
Pork (mean)	0.42	62

Adapted from Lombardi-Boccia, G., Martinez-Dominguez, B. & Aguzzi, A., 2002. Total heme and non-heme iron in raw and cooked meats. J. Food Sci. 67(5), 1738–1741.

TABLE 11.4 Total Iron Content and Percentage of Heme Iron in Cooked Poultry and Other Meats

	Total Iron (mg/100 g)	% Heme Iron
Chicken breast	0.58	28
Chicken thighs	1.34	22
Chicken wings	0.92	25
Turkey breast	0.79	27
Turkey thighs	1.46	39
Beef fillet	3.38	85
Veal fillet	1.58	83
Pork (mean)	0.64	61

Adapted from Lombardi-Boccia, G., Martinez-Dominguez, B. & Aguzzi, A., 2002. Total heme and non-heme iron in raw and cooked meats. J. Food Sci. 67(5), 1738–1741.

iron can also compromise the immune system, impair cognitive performance, and inhibit the ability to maintain a stable body temperature.

11.6 OTHER MINERALS

Sodium concentration is very low in fresh poultry meat, and does not significantly contribute to total dietary intake. On the contrary, processed meat products can contain high quantities of sodium, added as a preservative or flavor enhancer.

Red meat and poultry are the main dietary sources for zinc. Zinc is an essential trace element for humans (Maret, 2013), found in nearly 100 specific enzymes. It is the second most abundant transition metal in organisms after iron. In humans, zinc plays ubiquitous biological roles. It interacts with a wide range of organic ligands, and has roles in the metabolism of RNA and DNA, signal transduction, and gene expression. It also regulates apoptosis.

Symptoms of mild zinc deficiency are diverse. Clinical outcomes include depressed growth, diarrhea, impotence, and delayed sexual maturation, alopecia, eye and skin lesions, impaired appetite, altered cognition, impaired host defense properties, defects in carbohydrate utilization, and reproductive teratogenesis. Both mild zinc deficiency (Ibs and Rink, 2003) and excess zinc depress immunity (Rink and Gabriel, 2000).

Zinc content in poultry varies according to species and cuts, ranging from 2.68 mg zinc/100 g in turkey thighs without skin to 0.67 mg zinc/100 g in chicken breast (http://nut.entecra.it/646/tabelle-di-composizione-degli-alimenti.html).

The EFSA (2014a) has provided the estimated average requirements (AR) and PRI for zinc considering four different levels of phytates intakes (300, 600, 900, and 1200 mg/day), which cover the range of mean/median intakes observed in European populations. ARs range from 6.2 to 10.2 mg/day for women and from 7.5 to 12.7 mg/day for men. Accordingly, 100 g of turkey thighs provide 26%–48% of the daily zinc AR in women.

Poultry meat is also a good source of selenium. The selenium content in foods depends on the concentration of selenium in the soil where the crops were grown, and selenium deficiency is becoming more widespread in humans because soils are becoming depleted. Selenium is part of different selenoproteins, most of them acting as antioxidant enzymes.

The EFSA has considered the leveling off of plasma selenoprotein P (SEPP1) concentration not to be a sufficiently reliable marker to derive an AR of selenium, so only the adequate intake (AI) of 70 μg/day for adults was set (EFSA, 2014b).

Selenium content in chicken is about 10 μg/100 g, and in turkey 6–7 μg/100 g. Yu et al. (2008) showed that by adding 0.24 mg of selenium (as organic selenium) per kilogram of feed, the selenium content of breast meat increases from 8.6 to 41 μg/100 g, which is more than 58% of the reference daily intake (RDI). The same amount of selenium in the form of inorganic sodium selenite also increases selenium in breast meat, but only to 16 μg/100 g.

11.7 VITAMINS AND OTHER ESSENTIAL NUTRIENTS

Poultry, like other meat types, is the ideal dietary source of vitamin B_{12} and a good source of the majority of hydrophilic vitamins. While red meat is the most abundant in terms of vitamin B_{12}, poultry supplies an important amount of niacin.

Niacin, also known as vitamin B_3 and nicotinic acid, is one of the essential human nutrients. Insufficient niacin in the diet can cause nausea, skin and mouth lesions, anemia, headaches, and tiredness. Severe deficiency of niacin in the diet causes the disease pellagra, which is characterized by diarrhea, dermatitis, and dementia. Niacin can be synthesized in the human body from the indispensable amino acid tryptophan. Approximately 60 mg of tryptophan yields 1 mg of niacin defined as 1 mg niacin equivalent (NE). The PRI of niacin is 6.6 mg NE/1000 kcal (EFSA, 2014c). The niacin content of chicken breast (8.3 mg/100 g) is about 180% of niacin content in beef fillet (4.7 mg/100 g). Considering a 2000-kcal diet, 100 g of chicken breast provides about 63% of niacin PRI.

The amounts of other B-group vitamins (e.g., vitamin B_6, and pantothenic acid) in poultry are very similar to those in other meats and do not significantly diminish during cooking.

TABLE 11.5 Total Choline Content in Chicken and Beef

	Choline Content (mg/100 g)
Chicken, broilers or fryers, thigh, meat only, cooked, rotisserie	67.9
Chicken, broilers or fryers, breast, meat only, cooked, rotisserie	68.7
Chicken, broilers or fryers, drumstick, meat only, cooked, rotisserie	86.1
Beef, ground, 70% lean meat/30% fat, raw	46.8
Beef, ground, 80% lean meat/20% fat, raw	56.4

Data from the USDA National Nutrient Database for Standard Reference, release 28 (2015).

The concentration of lipophilic vitamins is lower in meat than in plant-based foods. Poultry meat can be enriched with vitamin E by providing it with feed (see Section 11.7).

Choline is an essential nutrient required for many metabolic processes including methyl group donation, lipoprotein metabolism, neurotransmitter synthesis, and cell membrane signaling (Zeisel, 2006).

The EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA) defines daily Adequate Intakes (AI) for choline as follows (EFSA, 2016):

- 400 mg for adults and adolescents aged 15−17 years;
- 140−340 mg for children aged 1−14 years;
- 160 mg for infants aged 7−11 months;
- 480 mg for pregnant women and 520 mg for lactating women.

Eggs, dairy, and meat are the major dietary sources of choline. Total choline (i.e., the sum of free choline, glycerophosphorylcholine (GPC), phosphocholine, phosphatidylcholine, lysophosphocholine, and sphingomyelin) content in chicken is higher than in beef (Table 11.5).

11.8 FUTURE PERSPECTIVES: POULTRY MEAT AS A FUNCTIONAL FOOD

It is traditionally recognized that chicken soup is a home-based remedy for colds and flu (Pachter et al., 1998); however, the scientific basis of this folk remedy is still lacking, since its functional efficacy has been proven only in an *in vitro* system (Rennard et al., 2000). Indeed foods can be considered functional or with beneficial impact on health only if there is evidence supporting their effectiveness (Clydesdale, 2004).

Functional foods have become a worldwide trend and a craving for healthier food and its market is continuously expanding based on increasing

consumption and interest (Goetzke et al., 2014). The development of meat and meat products as functional foods could not only improve the nutritional value, but also give a positive image of the products themselves (Jiménez-Colmenero et al., 2001; Olmedilla-Alonso et al., 2013). In this regard, improving the bioactive compound content in meat and meat products can be effective.

Important bioactive compounds in meat include coenzyme Q_{10}, taurine, conjugated linoleic acid (CLA), glutathione, lipoic acid, betaine, L-carnitine, creatine, carnosine, and anserine (Schmid, 2009). Previous studies have shown that the content of the mentioned functional compounds in meat is generally determined by several factors, including species, breed, age, sex, muscle type, and thermal processing (Alirezaei et al., 2012; Knüttel-Gustavsen and Harmeyer, 2011; Mora et al., 2010; Purchas et al., 2004). Poultry meat naturally contains a higher content of anserine than beef and pork (Abe and Okuma, 1995). However, unlike most other meats, poultry, and particularly chicken meat, can also be enriched with several important nutrients/bioactive compounds. Chicken meat with enhanced amounts of carnosine and anserine, which have high buffering and antioxidant properties, can be produced by high histidine feeding (Kai et al., 2015), and taurine contents of thigh and breast muscles increase linearly with increasing dietary taurine in broilers (Huang et al., 2014).

In monogastric animals, especially in chickens, it is well established that the fatty acid profile of feed directly affects the fatty acid composition of fat depots. CLA is a pool of isomers of the linoleic acid (octadecadienoic, C18:2), with conjugated double bonds. Cis-9-trans-11 C18:2 is the most important because of its biological activity. Recent findings point out its beneficial effect on human health, especially carcinogenic prevention, improvement of immunity response, and reduction of body fat content. A linear response has been proven between the CLA concentration in feed and the CLA content in poultry meat (Sirri et al., 2003).

Supplementation with small amounts of ALA increases the n-3 FA content in the form of ALA in chicken tissues, but it fails to increase the n-3 LC-PUFA content. On the other hand, the addition to feed of n-3 LC-PUFA (EPA and DHA) from marine sources is restricted due to organoleptic problems in the final product (López-Ferrer et al., 1999). In order to avoid organoleptic problems, a reduction of fish products in the diet during the last days before slaughtering has been tested. When linseed oil replaced fish oil for 1 or 2 weeks before slaughter, the sensory quality of the meat improved and maintained an important quantity of n-3 LC-PUFA (López-Ferrer et al., 1999). As reported in Section 11.3, the selection of specific genotypes having a higher desaturating activity may be a good strategy to increase EPA and DHA content in chicken meat (Boschetti et al., 2016).

Supplementation of poultry diets with vitamin E can achieve different objectives. In the first place, it prevents nutritional deficiencies in the

animals. Second, it improves the oxidative stability of broiler meat products. And, finally, it produces a highly nutritional food supplementary source of vitamin E for the human consumer. The α-tocopherol content in poultry meat increases linearly as the dietary vitamin E supplementation (level and administration time) increases (Bou et al., 2001), and vitamin E in thigh meat is 1.8- to 2-fold higher than in breast meat (Cortinas et al., 2006). But the vitamin E deposition in poultry meat is strongly affected by dietary PUFA content; as the dietary PUFA level increases, the α-tocopherol content of chicken meat decreases (Barroeta, 2007).

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Chapter 12

Ingredient Addition and Impacts on Quality, Health, and Consumer Acceptance

Shai Barbut
University of Guelph, Guelph, ON, Canada

12.1 INTRODUCTION

Nonmeat ingredients are used by all meat processors to achieve different tasks. The main ones include improved functionality (e.g., texture building by nonmeat proteins), flavor, appearance (color), and shelf life. In addition, the ingredients can be used to reduce formulation cost and enhance nutritional content/image (fiber addition). The discussion in this chapter will begin with the most common ingredients used in processed poultry meat products manufactured around the world. Overall, salt is the most often used ingredient because of its contribution to extracting the salt-soluble proteins from the muscle tissue (mainly actin and myosin), providing flavor, and suppressing growth of certain pathogens. Water/broth is another common additive which is added to many products and its quantity and quality are very important (e.g., low microbial count, no nitrite/sulfite contamination). Spices are also added to all/most products and provide unique flavors and colors but, in some cases, also used to suppress microbial growth (garlic extracts) and retard oxidation (rosemary). The discussion will continue with ingredients that are used to a lesser extent and include additives such as acidulants, hydrocolloid gums, enzymes, and smoke. Overall, these ingredients are used in products which have certain unique requirements (e.g., hydrocolloid gums in water added to an oven roasted turkey breast, breading on a chicken nugget).

Limits are imposed on the addition of several nonmeat ingredients while others are self-limiting (e.g., salt, garlic). The maximum amounts permitted can be found in local regulations/meat-inspection guides. An example of a restricted ingredient is nitrite, which is added to prevent the growth of deadly *Clostridium botulinum*. At high levels, however, nitrite can present a hazard

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and therefore the level is limited to 120−200 ppm in most countries. Other functional ingredients, such as soy protein, are also commonly regulated. For example, up to 3.5% soy can be added, alone or in combination with other binders, to a variety of sausages produced in the North America. However, if this limit is exceeded, the product name must include the words "soy added" or "imitation" to inform the consumer. In most countries, all the ingredients added to a food/meat product must be listed on the label so consumers can see what the product includes. In the case of some known allergens (e.g., nuts) and flavor enhancers (e.g., monosodium glutamate (MSG)), a specific warning may also be included on the label.

12.2 SALT

Various salts can be added to meat products. The most common salt is table salt (sodium chloride), which as indicated before, is used as a flavoring agent, protein solubilization agent, and antimicrobial agent. Phosphates are another group of salts used to help with meat protein extraction and solubilization (e.g., sodium tri-polyphosphate is used to help extract myosin and actin). Other salts include sodium nitrite (e.g., for preservation) and curing accelerators such as sodium erythorbate. It should also be mentioned that some consumers today look for reduced-salt products (Weiss et al., 2010; Anonymous, 2014) and this presents certain challenges to the food/meat industry.

12.2.1 Sodium Chloride

Sodium chloride (NaCl) is the most common ingredient added to meat products because of its three major contributions. The first and most important role is to help extract the salt soluble fraction (mainly myosin and actin), which is essential in the production of processed meat products. Overall, extracting the proteins and bringing them to the surface make them available to increase water-holding capacity (WHC), and provides sticky surfaces on raw pieces/chunks of meat to help "glue" the meat pieces together during cooking. These proteins also assist in emulsifying fat particles in comminuted products (by coating the fat globules), and increase the raw meat batter viscosity. Later, these extracted proteins coagulate and bind both the meat particles (important for holding the product together) and moisture (important to minimize cooking losses) to form a coherent matrix that is important for texture as well as fat retention during heat processing. Fig. 12.1 shows the effect of increasing salt concentration, in postrigor lean poultry meat, on the continuous decrease in product shrinkage, up to a maximum at around 5% salt. Further salt addition will result in increasing shrink loss, a phenomenon known as "salting out." This is the result of increasing charges on the protein molecules, which cause them to precipitate.

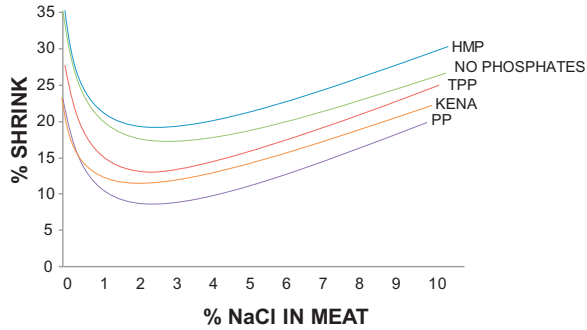


FIGURE 12.1 Effect of NaCl on the shrinking of cooked chicken muscle (70°C) in the presence of salt and different polyphosphates (0.5%). *HMP*, hexa meta phosphate; *KENA*, commercial phosphate blend; *PP*, pyro phosphate (sodium acid); *TPP*, tri poly phosphate. *Redrawn from Shults, G.W., Wierbicki, E., 1973. Effects of sodium chloride and condensed phosphates on water holding capacity, pH and swelling of chicken muscle. J. Food Sci. 38, 991.*

The second function of salt is to provide a distinct salty flavor. The classic salty taste is represented by sodium chloride and lithium chloride, whereas other salts usually have additional flavors associated with them which can include a mixture of sweet, bitter, sour, and salty. Chemically, it appears that cations cause salty tastes, whereas anions inhibit salty tastes (Sebranek and Bacus, 2007). Among the anions, chloride is the least inhibitory to the salty taste and does not possess a taste of its own. Some anions can not only inhibit the taste of their associated cations, but also contribute tastes of their own. An example is the soapy taste associated with certain phosphates, which results from the specific taste elicited by their anion. In general, the most accepted model for describing the mechanism for salty taste perception involves the interaction of hydrated cation−anion complexes with the Shallenberger and Acree AH/B-type receptor site. The individual structures of such complexes vary substantially. In the presence of water, OH groups and salt anions/cations are associated with specific receptor sites. Bitterness in salts involves a different receptor mechanism that seems to be related to the sum of the ionic diameters of the anion and cation components of the salt. Salts with ionic diameters below 6.5 Å are salty in taste (LiCl = 4.98 Å, NaCl = 5.56 Å, KCl = 6.28 Å), although some individuals find KCl somewhat bitter. As the ionic diameter increases, salts become increasingly bitter (CsCl = 6.96 Å, CsI = 7.74 Å, $MgCl_2$ = 8.50 Å).

The third function of sodium chloride added to meat products is to suppress microbial growth, as many microorganisms are sensitive to high salt levels. High salt concentration can stop or substantially slow the growth of microorganisms. In the past, high salt levels (10%−20%) were used as the main means of preservation because these levels can provide shelf-stable meat products. This technique is still used in places where mechanical

refrigeration is lacking and/or where the traditional heavily salted products are preferred. Note that the very high salt content has to be washed out before consumption (Sebranek and Bacus, 2007). However in many markets today, substantially lower salt levels are used (e.g., 1.0%−2.5%), and it is only in conjunction with other additives (e.g., nitrite, lactic acid) and appropriate refrigerated storage that product safety can be ensured (Barbut and Findlay, 1989).

Salt reduction (mainly sodium) in food/meat products was a hot topic in the 1980s and is again today, as more individuals are suffering from hypertension (high blood pressure). It is estimated that salt consumption the Western diet is 5- to 10-times higher than the daily recommended level. An important consideration in replacing NaCl with other chloride salts is the effect on the physical properties of the final product. Sodium chloride reduction by itself will result in lower binding and lower WHC of the proteins. Upon heating, this will result in a softer, drier product with higher cooking losses. Overall, if cooking losses are too high the product will be unacceptable to the consumer. The relationship between salt concentration and WHC has been well established (Fig. 12.1) and also depends on factors such as the quality and type of protein extracted, pH, and previous storage history of the meat (e.g., frozen storage).

12.2.2 Sodium Nitrite and Sodium Nitrate

Sodium nitrite ($NaNO_2$) and sodium nitrate ($NaNO_3$), also known as curing salts, are added at low levels (usually 120−200 ppm) and provide several attributes. The main function is to suppress *C. botulinum* spore germination. The active compound is nitric oxide (NO). Only a very small amount is needed and using the salt form provides an easy and efficient way of introducing the active compound to the meat (i.e., NO gas can be introduced as well, but it is a much more expensive process).

The second function is to help develop the typical pink cured-meat color. Again, the active compound is NO. This pink color is very different from the brown color of a cooked product such as chicken/turkey leg meat. This can be described as the difference between home-cooked turkey dark meat (e.g., thigh meat) which is brown and a turkey ham product which appears pink. This is a reaction between the myoglobin and NO which initially forms nitrosomyoglobin and later with heat, the stable nitrosohemochrome. The third function is to help protect against lipid oxidation. Nitrite has antioxidant capabilities that can help prolong the shelf life of cooked meat products which are sensitive to oxidation.

Adding nitrite also helps in the development of some unique flavor notes. The amount of nitrite permitted in meat products is heavily regulated because at high levels it can be toxic. It is very important to note that processed meat products are not necessarily a high source of nitrite in our diet.

In comparison, green vegetables such as celery have levels of about 300 ppm nitrate. In addition, bacteria in human saliva and in the gut are capable of producing even higher levels of nitrite. Nitrite added to meat products is depleted over time, especially during cooking, and a frankfurter with an initial 150-ppm $NaNO_2$ level will end up with about 20−40 ppm or less at the point of purchase. Overall, it is estimated that meat products contribute only 10%−20% of the total nitrite in our diet (Sindelar and Milkowski, 2012). There is also a concern in products heated to high temperatures (e.g., bacon) that residual nitrite could react with secondary amines to form nitrosamine compounds, which are potential carcinogens. Therefore, in North America for example, the use of an added curing accelerator (e.g., 500 ppm ascorbate) has been mandated in such products to ensure a fast conversion of nitrite to nitric oxide. This minimizes the chance of nitrosamine formation when the product is exposed to high temperatures (frying at $>100^{\circ}C$). The use of nitrite in processed meat products and its safety has been reviewed by Cassens (1990), Honikel (2008), and by Sindelar and Milkowski (2012).

12.2.3 Phosphates

Salts of phosphoric acid can work together with sodium chloride to enhance muscle protein extraction, which in turn improve the WHC and reduce shrinkage during cooking (Fig. 12.1). There are many different phosphates available on the market. Alkaline polyphosphates such as tri-polyphosphate (TPP) are the most popular and by some estimates account for about 80% of the phosphates used by the meat industry. Phosphate use is limited to 0.5% in the finished product in countries such as the USA. This limit is mainly imposed to restrict water addition but greater levels can also result in off-flavor problems such as metallic or soapy as reported by consumers. In countries such as Germany, the use of phosphate is not permitted in several products on the market.

Fig. 12.1 demonstrates the effects of using 0.5% TPP, pyrophosphate, and a commercial blend called KENA (contains over 50% TPP). A synergistic effect is clearly seen when TPP is used with 2%−5% salt; i.e., the combined effect of NaCl and TPP is much greater than the simple additive effect of NaCl and TPP. The "salting out" effect, previously discussed, is clearly seen at a salt level above 5%. Pyrophosphate and NaCl show an even greater synergistic effect compared to TPP (Fig. 12.1) but pyrophosphates are not commonly used due to their effect on pH and other factors. Hexametaphosphate, for example, results in higher shrinkage during cooking. This raises the point that processors should know exactly what kind of phosphate(s) or blend they are using. Overall, many of the phosphates used by the meat industry help enhance the physical and sensory properties of meat products by helping extract the salt-soluble proteins, hence increasing WHC and meat particle binding. Many phosphates can shift the pH away from the isoelectric point of the muscle's proteins, hence allowing more charges on the amino acid side

chains. This helps increase repulsion between the proteins, which creates more spaces for water molecules and more sites for water molecule binding. Various researchers including Lee et al. (2011) and Petracci et al. (2013), demonstrated the beneficial effect of using sodium tripolyphosphate to enhance WHC and tenderness of marinated whole muscle poultry meat products.

It is important to note that since phosphates have a molecular structure that contains hydrophilic/hydrophobic sites, they assist in stabilizing meat emulsions. They also slow down oxidation due to the chelating effect of phosphate. Certain phosphates can bind iron and other metals and prevent them from acting as pro-oxidants. This helps extend the shelf life of the meat product in terms of flavor and also protects it from meat pigment oxidation (color fading).

12.2.4 Sodium Ascorbate and Sodium Erythorbate

Sodium ascorbate and sodium erythorbate, also known as curing accelerators, are added to increase the rate at which nitrite is reduced to nitric oxide in a meat system (some of the muscle's enzymes also help reduce the nitrite). However, in order to speed up the conversion to nitric oxide, curing accelerators that promote reducing conditions are added. In addition, they also accelerate the reduction of metmyoglobin to myoglobin which is especially important in continuous sausage production lines, where the processor's objective is to start cooking the product within an hour of blending. In other cases, such as the manufacture of dry sausages, a slow nitric oxide release is preferred and curing accelerators are not needed. In these cases, processors are actually using sodium nitrate, which takes even longer to breakdown to nitric oxide than sodium nitrite, so they get a prolonged release of nitric oxide.

Sodium ascorbate and sodium erythorbate or their corresponding ascorbic acid and erythorbic acid are used at low concentrations of about 550 ppm. For products that will be exposed to high-temperature cooking (e.g., turkey bacon), a number of countries require that a curing accelerator be added as high temperatures can increase nitrosoamine formation as mentioned above.

12.3 WATER

Water is usually used to increase juiciness, compensate for evaporation losses during cooking, and as a system to reduce fat content in products. The latter reflects the trend of combining water, hydrocolloid gums and/or starch to mimic the fat mouthfeel. The amount of added water is regulated in many countries and if the moisture added exceeds a certain level in the finished product (i.e., after cooking and taking into account evaporation loss), it should be mentioned in the product's name (e.g., Chicken Roll with Natural Juices/Water Added). Overall, water is the main component in fresh and processed meats, ranging from 40% to 80%. Most of the moisture originates from the lean meat portion (e.g., skinless poultry breast meat has 75% moisture). Industry and

consumers add moisture to products because otherwise they would have a dry mouth feel. An example is a chicken fillet cooked with an initial 22% protein but ending with about 25% protein after cooking and taking into account evaporation loss. This product will be fairly tough and unacceptable to most consumers. Added moisture increases product acceptability by compensating for moisture lost during the heating process. Added moisture also serves as a carrier for spices and other nonmeat ingredients and ensures their adequate distribution. In finely comminuted meat products, ice is added during chopping to maintain a sufficiently low temperature so that heat arising from the high friction during cutting will not cause an emulsion breakdown.

The microbiological and chemical quality of the water is an extremely important issue. From a microbiological standpoint, water should meet the drinking-quality standards (e.g., municipal, national standards) at the very least and should be checked on a regular basis. From a chemical standpoint, water contaminated with compounds such as nitrate will cause undesirable pinking of products such as oven-roasted chicken breast. Nitrite contamination (1−5 ppm) can be common in agricultural areas where the water source is located near fields that are fertilized with nitrates. Another consideration is the presence of high salt levels. Water with calcium and magnesium salts, also referred to as hard water, can destabilize emulsion-type meat products as well as cause problems within a plant's piping system.

12.4 SPICES, SWEETENERS, AND FLAVOR ENHANCERS

These nonmeat ingredients are added to most/all meat products to add unique flavors and aromas. The use of spice usually reflects a certain ethnic/national tradition.

12.4.1 Spices

Spices are used to color foods (e.g., paprika) as well as to add some antimicrobial/antioxidant (e.g., rosemary) properties (Radha-krishnan et al., 2014). In several cases they are also used to enhance appearance (e.g., peppercorns on barbequed meat).

Examples of spices derived from different plant materials (Fig. 12.2) are listed below.

- *Bulbs*: onion (*Allium capa*)
- *Seeds*: mustard (*Brassica nigra*)
- *Fruit*: paprika (*Capsicum annuum*)
- *Leaves*: sage (*Salvia officinalis*)
- *Roots*: ginger (*Zingiber officinale*)
- *Flowers*: clove (*Eugenia caryophyllata*)
- *Bark*: cinnamon (*Cinnamomum zeylanicum*)



FIGURE 12.2 Spices that can be used as bacteriostatic agents in food preparation. Showing here black pepper corns (right) and ground pepper at different granulations.

Spices can be added in different forms depending on the product, desired appearance, expected shelf life, etc. In most commercial applications they are added dried or after a heat treatment because they are then easier to handle (e.g., longer shelf life, inactivated enzymes that can produce off-flavors/colors in the meat) and easier to standardize (e.g., strength/heat of pepper). Overall, spices can be added before or after being dried (e.g., onion), whole or ground (e.g., black pepper, mustard seeds), or as extracts (e.g., rosemary oleoresin). The decision as to which form is used is based on the meat product type and desired appearance. In coarse-ground products such as salami, whole mustard seeds can be added so the cross section of the product has a nice appearance. However, in a finely comminuted frankfurter, whole mustard seeds would not be as attractive.

Spices commonly carry a high number of microorganisms. Therefore, they should be thoroughly cleaned and pasteurized/sterilized. Nonheat processes such as ionizing irradiation and chemical pasteurization (e.g., ethylene oxide) are commonly used. Both are considered cold processes that do not volatilize the flavor/aroma compounds. Ionizing radiation is used quite extensively and usually irradiated spices do not have to be identified as irradiated in the meat product ingredient statement, because of the low level of addition. If the whole product is irradiated, however, certain countries require that the international logo for irradiated food appear on the label. Chemical sterilization processes are also commonly used but some consumer groups are concerned with the potential risk of residues.

Large volumes of spice extracts, which contain essential oils and oleoresins extracted from plant material, are sold to the meat industry. The oils can be obtained by pressing, distilling, or solvent extraction, and they are usually

concentrated to obtain a more potent solution. The oils will be free of microorganisms if a high distillation temperature or a strong solvent is used. Overall, the advantages of using oil extracts include reduced transportation costs, long shelf life, and they do not change the appearance of the product. In finely comminuted products such as hot dogs and bologna, which have a very homogeneous appearance, this latter point is very important (e.g., adding visible ground black pepper particles would not be acceptable to the consumer). The extracts are usually highly concentrated and are commonly sprayed on to a carrier such as salt or sugar (dextrose) in order to ensure an even distribution within the product (Barbut, 2015).

Standardizing the flavor strength is an important consideration when using natural spices or extracts. Spice companies purchase materials from around the world and factors such as growing conditions, climate, and plant variety can result in large variations in taste. To overcome this, spice companies employ trained personnel to standardize flavor profiles and obtain defined strengths (e.g., determine the Scoville Heat Unit for red pepper). This is extremely important to meat processors who want to create a consistent product. When standardizing the flavor of a spice, a technician prepares serial dilutions of the extract and presents them to a trained panel to identify the lowest concentration the panel can detect. This lower threshold can be used to standardize the flavor. Additional sophisticated equipment such as a gas chromatograph can also be used to determine the concentrations of key flavor compounds that contribute to the overall flavor of a spice. In the case of color standardization of a spice such as paprika, the red color intensity can be described by the scale developed by the American Spice Trade Association (i.e., measuring absorbance of a sample diluted in acetone at 460 nm).

12.4.2 Sweeteners and Browning Agents

Sweeteners and browning agents are ingredients commonly used in low amounts to add a sweet flavor, mask saltiness, enhance browning (i.e., the Maillard reaction), and provide a substrate for fermentation.

Overall, natural and synthetic sugars vary in their sweetness. The standard for the measurement is sucrose, which is assigned a sweetness value of 100. On that scale, dextrose has a value of 74, fructose has a value of 175, maltose has a value of 40, lactose has a value of 16, regular corn syrup solid has a value of 37 (therefore it can also be used as a partial bulking agent; see discussion below), and aspartame has a value of 18,000. There is a wide selection of sugars to choose from and most commonly the meat industry uses natural sugars at about 1%−3%. The reason for adding the sugar often determines the type used. Reducing sugars contribute to the Maillard browning reaction when they combine with secondary amines to form a brown pigment during heating. Adding a reducing sugar such as dextrose, to a meat product will enhance surface browning. This is important in smoked

sausages where a golden/brown color is desirable. Reducing sugars can be also added to fried products where adequate golden/brown color development during heating prevents overcooking/burning the sausage.

12.4.3 Flavor Enhancers

Flavor enhancers are compounds that act synergistically with meat flavor compounds to enhance the meaty flavor. A few of the most commonly used ones are 5′-ribonucleotides, hydrolyzed yeast proteins, and MSG. When used at levels in excess of their independent detection thresholds, these compounds contribute to what is called the delicious or umami taste of foods. When used at levels below the independent detection threshold they simply enhance flavors. It is important to recognize that a very small percentage of the population is sensitive to ingredients such as MSG, and therefore, MSG should be clearly marked on the package. Overall, one should remember that meat product's flavor can be affected by many factors (e.g., animal, breed, feed used, aging after slaughter, cooking method) and their interactions (see review by Khan et al., 2015).

12.5 ACIDS AND ACIDULANTS

Acids and acidulants are used to reduce pH, add flavor, extend the shelf life and/or produce a fermented-like meat product. A common example of an acid is vinegar and an example of an acidulant is glucono delta lactone (GDL), which can yield a more rapid color development to cooked comminuted meat products. An important advantage of using GDL is its slow acid release which does not cause a problem with later protein binding. Although GDL was introduced to accelerate raw meat processing operations, it has more recently been used in the production of fermented-like meat products.

Using encapsulated acids is another way to add acids (e.g., lactic, citric) to produce a fermented-like product. The encapsulation material is usually made of hydrogenated vegetable oil that melts at around 65°C.

12.6 BINDERS

Binders are ingredients used to help "glue" meat particles and increase WHC. These ingredients usually consist of proteins that can form a gel system or participate in meat protein gelation. It is advantageous if they act synergistically with the meat proteins (Fig. 12.3). These ingredients can be expensive so when processors consider using them they should look for added values such as: reducing shrinkage during processing, texture enhancement, emulsification capabilities, and reduction of formulation cost.

The meat industry commonly uses vegetable proteins, dairy products (caseinate, whey), and meat proteins (collagen, blood plasma).

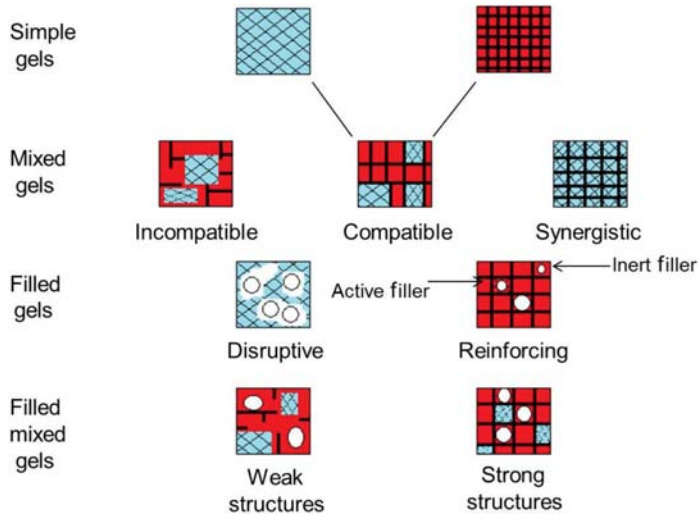


FIGURE 12.3 Possibilities for engineered structures of simple, mixed, filled, and filled—mixed gels. *Redrawn from Aguilera, J.M., Kessler, H.J., 1989. Properties of mixed and filled type dairy gels. J. Food Sci. 54, 1213.*

Examples of vegetable protein binders include soy proteins—commonly used as binders in products such as meat patties, meat loaves, and sausages. In a number of countries their presence is limited to $\leq$2% soy protein isolate or else the product name should include the word "soy." Other vegetable proteins such as pea are also used, but to a lesser extent. The vegetable proteins are commonly marketed under certain categories: (1) flour—fine particles with 40%−60% protein content; (2) concentrates—with 70% protein; (3) isolates—with 90% protein.

12.7 FILLERS

Fillers are nonmeat ingredients, usually consist of complex sugars (e.g., starch) and low protein, that help bind water but not meat particles and are usually considered to be good as bulking agents. They can be divided based on their cereal source (wheat, corn starch) and are added to the meat product either as flours or extruded/texturized particles. When starch is heated past its gelatinization temperature, in the presence of water, it opens up to bind water (e.g., can be 1:2 up to 1:10 ratio). At high temperatures the solution becomes more viscous and when temperature is lowered (e.g., cooling the food) the texture will become even more viscous. The meat industry also uses pregelatinized starches where the starch manufacturer has heated the product (in a solution) and then dried it. This creates a product that is capable of binding water at lower temperatures, which is advantageous for the

meat industry because as meat proteins are heated (and denatured) they bind less water. See additional explanation concerning the chemical structure of flour in Section 12.9.

12.8 GUMS

Gums are unique compounds that are capable of forming a high water gel matrix at low concentrations where, for example, a 1% carrageenan forms a very firm gel after heating, and binds 99% water (Venugopal, 2011). Such gums are added to meat products at relatively low concentrations to bind added brine/water. In this case the firm gel (upon cooling) also helps enhance the texture. Many gums are obtained from seaweed, some are extracted from seeds, and others are the product of microbial fermentation. Below are a few examples of common hydrocolloid gums.

12.8.1 Carrageenan

Carrageenan is a gum that is extracted from Irish moss (*Chondrus crispus Stackh.*). It is composed of monomers of sulfated galactose and anhydro-D-galactose. The gum is a complex mixture of about 10 different polymers. The main ones used by the meat industry are kappa and iota. The type of gel formed depends on refining the raw material, the dominant polymer in the mixture and the cation used to induce gelation during heating. Carrageenan forms a reversible gel (i.e., can be remelted) is very effective at binding water, and is sometimes added to products where water is used to replace fat such as oven-roasted turkey/chicken breast products and low-fat sausages.

12.8.2 Alginate

Alginate is extracted from brown algae (*Phaeophyta*). Alginate is composed of manmuronic and guluronic acid monomers; the ratio between them determines the brittleness of the gel, water holding, etc. Since the algae are harvested at different places and during different seasons, there are variations in the gelling performance. Therefore, it is important that the meat processor uses a supplier who is reputable and can control and standardize the gel performance (similar to spice strength, previously discussed). One of the unique characteristics of alginate is its ability to gel at low temperature when a small amount of calcium ions is added. The meat industry uses it for binding raw meat particles in products such as restructured steaks/patties in order to provide binding of the smaller meat trimmings and hold the product together prior to cooking. It is also used today to make casings that are co-extruded directly onto the product (Harper et al., 2013).

12.8.3 Xanthan Gum

Xanthan gum is commonly obtained by microbial biosynthesis and is an extracellular polysaccharide. It is composed of cellulose chains with attached oligosaccharide groups. Low xanthan concentrations produce a highly viscous solution. Together with locust bean gum, xanthan can produce a thermo-reversible gel in various meat products (Venugopal, 2011).

12.9 BATTER AND BREADING INGREDIENTS

Batter and breading ingredients are used for coating various poultry products (bone-in chicken wings, boneless fillet strips, nuggets). Fig. 12.4 shows battered and breaded chicken tenderloins produced with different types of breading materials.

The description of the ingredients used will follow the sequence of the process in which they are applied to the product. The steps are basically composed of predusting, applying a semi-liquid batter, followed by breading and frying. This process can be repeated a few times if the processor is interested in increasing the amount of pick up (Barbut, 2015).

12.9.1 Predusting

Predusting is used to cover the meat with a fine coat of flour, as the first layer before batter and breading are applied. However, it should be noted

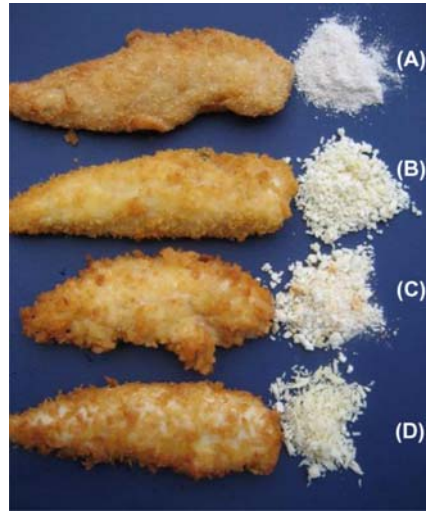


FIGURE 12.4 Examples of the four main categories of breading used to coat food products. (A) flour; (B) cracker type; (C) American/home-style; (D) Japanese style. *Photo by S. Barbut.*

that it is not used in all products and the decision to apply predust depends on factors such as the wetness of the surface. The predust adheres to the surface by absorbing free water on the surface and this is used to form a mediating layer between the product and the next batter layer. Enhancing predust adhesion can be achieved by supplementing the dry mix with proteins such as soy. Since the predust layer is the first coat on the product, it is often used to carry seasonings and spices (see additional discussion in Section 12.4), as they are going to be most protected during the frying and/or heating operation. This is advantageous because flavor components are fairly volatile and embedding them under the batter and breading layers can assist in protecting them.

12.9.2 Battering

Battering is used to coat the product with a wet solution, which consists of a suspension of dry ingredients (flour, starch, proteins) used to create the base for adhering the next layer of dry breadcrumbs. The industry uses different types of batters which can be separated into three main categories, based on viscosity, solid content, carbohydrate, and/or protein source

12.9.2.1 Adhesion Batters

Adhesion batters are designed to adhere to the meat product and are usually starch based with a high solid content and low viscosity. The main ingredient is corn starch or modified corn starch. The batter is commonly applied as a relatively thin coat and is important that it dries fairly quickly, so that a significant amount stays on the product. The common ratio of dry ingredients to water is about 1.0:1.6. Online viscosity measurement is an important quality-control tool as it can assist in monitoring the process. Manual viscosity determination is common where either a rotational viscometer or a simple funnel is used to determine the time it takes for a specific volume of batter to flow through a narrow opening. The latter is a fast and very inexpensive way and is known in the industry as Stein cup measurement. For adhesion batters which are considered low viscosity, batter flow values are typically 8−12 s at refrigerated temperature.

12.9.2.2 Cohesion Batters

Cohesion batters are used to form an envelope/shell around the meat product, and as a base for attaching the next breading layer. These batters are thicker than adhesion batters and are usually flour based. They contain a medium amount of solids and are also used to provide texture to this layer. The drying time is longer than for adhesion batters, but since they are more viscous (28−30 s, as measured by the Stein cup), they will not run off as quickly. The common dilution of dry ingredients to moisture is 1.0:1.9.

12.9.2.3 Tempura Batters

Tempura batters are usually not supplemented with breading, and are used to create a puffed and crispy layer around the product. Overall they can be seen as cohesion batters which include a significant amount of leavening agents. Tempura batters are usually made from a mixture of flour and starch, and have a high solid content. This results in a high viscosity of the batters of about around 45 s in the Stein cup. The batter is designed to have good cohesive characteristics and as a result of the leavening ingredients, forms a layer rich in air pockets. The inclusion of leavening agents makes the batter sensitive to over-mixing and pumping. A high degree of mixing/agitation will result in a fast release of the gases which are supposed to be released during the frying operation. Overall, it is recommended that the product be fried very soon after applying tempura batter. The common dilution in tempura batters of dry ingredients to water is 1.0:1.0.

As indicated above, the main ingredients found in a batter are wheat flour, corn flour, proteins, gums (see separate discussion Section 12.8), and leavening agents (most common is sodium bicarbonate). Wheat flour is obtained from finely ground wheat's endosperm and contains both carbohydrates and proteins. The carbohydrate component, mainly consisting of starch, is useful in providing good adhesion to the product. Modified starch can be used to further enhance adhesion. Starch also contributes to the drying time of the batter and the development of a crispy texture during the frying operation. In some batter applications, the ratio of highly branched starch polymers (amylopectin) and linear polymers (amylose) has a profound effect on the starch functionality. The ratio is quite variable and ranges from 99% amylopectin in waxy rice, to high amylose content in cornstarch. Overall, starch molecules absorb water during batter preparation, and some starches hold water better than others (e.g., modified starches). The type of starch can have a strong effect on the batter's viscosity. For example, Xue and Ngadi (2006) studied the viscosities of batters prepared with blends of wheat and corn, wheat and rice, and corn and rice. In the case of wheat and corn, the viscosity decreased faster when a higher proportion of corn flour was used (viscosity decreases with increasing shear rate), suggesting that it diluted the strengthening influence of the wheat flour gluten. Rice flour also exerted a diluting effect on wheat gluten, and as suggested by the authors, increased the available free water in the batter system. This free water could lubricate particles, enhance flow, and result in lower viscosity values. Overall, the addition of corn flour caused greater viscosity reduction than rice flour.

12.9.3 Breading

Breading provides a unique appearance, and texture, and increases the volume and weight of the product. The type of breading can range from a

simple flour, to structured baked crumbs. Usually, the breading is a cereal-based product which has been baked and later ground into fine (>60 mesh), medium (20−60 mesh), or large size crumbs (up to 20 mesh size). In addition, ingredients like sesame seeds can also incorporated. The dry breading material adheres to the product via the sticky batter, and therefore, it is important to match the batter with the right breading. The ingredients that are used to make the breading include typical components used for bread preparation and include flour, water, salt, sugar, yeast, etc. There are many products on the market but they can be divided into four major groups (Fig. 12.4).

12.9.3.1 Flour

Flour can be used in its raw form as an economical way of coating a poultry meat product. The resulting fried coating provides relatively low surface browning and a very dense coating matrix.

12.9.3.2 Home-Style/American Bread Crumbs

Home-style/American breadcrumbs resemble the type of crumbs consumers can prepare at home. The crumbs come in different sizes and provide a distinct crust and attractive highlighting during the frying operation—a medium to high browning can be achieved. It has a more open structure compared to the flour or the cracker-type crumb which results in a more crispy texture of the fried product.

12.9.3.3 Traditional/Cracker-Type Crumbs

Traditional/cracker-type crumbs are usually white or colored breadcrumbs, with minimal or no crust on the surface. This relatively inexpensive crumb with a flat, flake-like structure is easy to use and provides an even surface on the coated product. The browning, achieved during the frying operation, is considered low and the crumbs can be used for full-fry or oven-heated type products. The flakes themselves are fairly dense and give the final product a crunchy texture.

12.9.3.4 Japanese-Style Crumbs

Japanese-style crumbs have a very defined shape which resembles an elongated spindle/shredded cheese. Since the delicate, three-dimensional structure is fragile, special equipment with minimal friction should be used for its application. The texture of the crumb is fairly open/porous (see Fig. 12.4) and it is produced as white or colored material. The crumbs are commonly produced by an electrical induction heating process, rather than conventional baking. Because of the light structure, it is possible to produce a large-sized crumb without the sensation of hard particles that is prevalent in other crumbs.

12.9.3.5 Oil

Oil is another ingredient that is added/absorbed by the product and its breadcrumbs, during frying. In general, porous structures (e.g., Japanese-style crumb) will absorb more oil than dense structures (cracker-type crumb).

The frying operation is used to cement the soft coating system (so it will stay on the product), to develop a brown/gold color on the surface, to provide a unique texture, and inactivate pathogenic microorganisms. The different oils/fats commonly used can be divided into: vegetable and animal fats. Using one type of oil versus another depends on factors such as cost, consumer preference, stability, and flavor. In terms of products' quality, the increase in viscosity of the oil over time can result in higher oil pick-up and an insufficient heat transfer. In most conventional operations, the oil is continuously filtered to remove charcoal particles (e.g., fall off breadcrumb) and fresh oil is added to replace oil absorbed by the products (i.e., about 10% of the oil is absorbed by the passing products). Overall, oil turnover, coupled with constant filtering, is usually sufficient to run an adequate continuous frying operation (Barbut, 2015).

12.10 ANTIOXIDANTS

Antioxidants are important compounds used to suppress lipid oxidation. This is a critical issue in fresh meat and meat products as animal fat is prone to lipid oxidation. In addition, there is disruption of cells during processing (e.g., cutting), and enzymes are released during processing, heat treatments, and prolonged storage, which all can further induce lipid oxidation. In living tissues there are various natural antioxidants such as tocopherol; however, they are not always sufficient to protect the meat/meat products after processing. The process of oxidation is driven by free radical formation and, once started, it accelerates exponentially. The food industry uses three types of antioxidants. The first is free radical terminators, the second is oxygen scavengers, and the third is chelating agents capable of tying up metal ions.

Because governments have certain restrictions on using synthetic antioxidants and some are not permitted, natural antioxidants such as rosemary oleoresin are an attractive option and are also label friendly. In this case, they are only listed by the spice name (Radha-krishnan et al., 2014). Products such as a low-flavor rosemary oleoresin are also available, where most of the flavor compounds have been removed so that the amount of the oleoresin added to the product can be increased. Citric acid is an example of a molecule that is an oxygen scavenger.

The industry also uses synthetic antioxidants such as butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), and propyl gallate (PG). These compounds are free radical terminators, as they have a cyclic carbon ring structure that is capable of accepting a free radical molecule. These

compounds are fat soluble and their usage level (where permitted) is commonly limited to 200 ppm of the fat content. Barbut et al. (1985) showed the beneficial effects of using a BHA/BHT mixture (200 ppm) and natural rosemary oleoresin on delaying lipid oxidation in a turkey sausage produced with 25% mechanically deboned meat, which is highly susceptible to oxidation. Both the mixture and the rosemary oleoresin were very effective in suppressing oxidation in the stored product. The spice mix used in isolation also resulted in some antioxidant activity as compared to the meat control treatment. The authors also illustrate the effects of salt, which can accelerate lipid oxidation due to a small amount of heavy metal contamination. The data was reported as the amount of malonaldehyde (i.e., an oxidation byproducts from the breakdown product of oxidized fatty acids), which is commonly used in the literature to follow lipid oxidation. The publication also listed the amounts of other byproducts (e.g., hexanal, heptanal, penanol; measured by gas chromatography) that contribute to off-odors that can be detected by a sensory panel. Rocío et al. (2015), also showed the beneficial effect of different rosemary extracts on frozen chicken nuggets (i.e., sensitive to oxidation due to the high-temperature frying process used prior to freezing and distributing the product).

12.11 ENZYMES

Several groups of enzymes can be added to meat products for a variety of reasons. The two main groups are used for binding meat particles/surfaces and for tenderizing tough meat cuts. Transglutaminase is an example of commercially available enzyme used to bind meat pieces at low temperature (i.e., prior to cooking) in products such as restructured steaks/patties. This specific enzyme has been used for hundreds of years in the production of fish surimi, although the chemistry was not understood until recently. Transglutaminase is able to catalyze acyl transfer reactions and introduce covalent cross-links between proteins (Weiss et al., 2010). It is now commercially harvested from microbial fermentations. Cofrades et al. (2011) reported that adding transglutaminase to low-salt restructured poultry steaks significantly increased shear force of the raw products making them easier to handle.

The second group of enzymes used is the one that can break down connective tissue. Papain and ficin extracted from pineapples and figs, respectively, are able to break down collagen and are sometimes used to tenderize meat. However, their activity should be stopped at a certain point as extensive proteolysis can turn the meat into mush.

12.12 SMOKE COMPOUNDS

Natural and liquid smoke are mainly used to provide flavor, color (Maillard reaction), antimicrobial protection, and antioxidant compounds to the surface of the product. In the past, smoking of poultry meat cuts was carried out

over an open fire for an extended period of a few days in order to preserve the products (including drying). However, today, smoking is usually done for about 1 h and is mainly done to add flavor and color and to help increase the shelf life to the product (see review by Lingbeck et al., 2014).

There are several hundred different compounds in natural smoke. Toledo (2007) reported over 300 in some of the commonly used hardwoods. The compounds can be divided into four main groups which include:

- organic acids—help in preservation and coagulate surface proteins;
- phenols—contribute to color, flavor, preservation, and antioxidation;
- carbonyls—contribute to flavor and color development;
- polycyclic hydrocarbons—created when high burning temperatures are employed; some of the compounds, such as benzopyrene, are potentially carcinogenic.

Smoke can be applied by burning the sawdust or pieces of hardwoods (e.g., maple, cherry) or as a liquid smoke solution. The former is achieved with the help of a special generator outside the smokehouse. As the moist sawdust is slowly burned, the smoke is circulated into the smokehouse by a fan system. During this process, the exhaust duct should be closed so smoke can accumulate and is not wasted. Liquid smoke is prepared in dedicated facilities where smoke compounds from burning wood are captured by letting the smoke rise inside a tall chimney equipped with a counterflow water shower. The smoke compounds can later be concentrated and the preparation is applied to meat products as a dip, spray, or atomized mist. In addition, some liquid smoke products can be added directly to the raw product after the pH has been adjusted.

12.13 MOLD INHIBITORS

Mold inhibitors are used to inhibit growth on the surface of dry and semi-dry sausages that are not vacuum packed. This can be a problem as these products have a water activity that supports mold growth. Mold inhibitors are applied by dipping or spraying the outside casings. Common chemical inhibitors include potassium sorbate and sorbic acid. These compounds are permitted for use in some countries, but not others. In countries where these compounds are not permitted, processors can use a cold smoke treatment that contains natural antimicrobial compounds that help prevent mold growth on the surface.

12.14 STARTER CULTURE

Bacteria capable of producing lactic acid are added to fermented sausages, such as salami. *Lactobacillus plantarum* and *Pediococcus acidilactici* are used to ferment the added sugars and produce lactic acid, which decreases the pH of the product. This helps to make the product shelf-stable and

provides unique flavors and textures. Processors should add a simple sugar source to prevent bacterial utilizing of fat and proteins which will result in the formation of oxidized compounds and putrefied odors. In the past, processors relied on the naturally occurring lactic acid bacteria or an innoculum from a previous batch of products for the fermentation. Today, however, many use a standardized, controlled starter culture produced by specialized companies. Altera et al. (2006) demonstrated the inhibitory effect of starter culture on *Campylobackter jejuni* in German-style fermented turkey sausages and indicated that the greatest effect was during the first 4 h of ripening; i.e., when pH is quickly dropped.

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Chapter 13

Consumer Attitudes to Poultry Meat: A Comparative Study of the UK and China

Patricia Parrott and Keith Walley
Harper Adams University, Newport, United Kingdom

13.1 INTRODUCTION

According to the Food and Agricultural Organization (2015) poultry grew from 13% of the global meat sector in the mid-1960s to 28% in 2015. Although there are significant differences in the amount of poultry meat consumed, for instance in 2014 poultry meat consumption in a developed country such as the USA was on average 45.6 kg per person while in a developing nation like Mexico it was only 26.3 kg per person, average consumption has risen from 11.0 kg per person per year in 2000 to 14.4 kg in 2011 (Evans, 2016). Currently, chicken is the most widely consumed meat at around 89% of poultry meat availability and by 2024 chicken production could grow to reach 118 million tonnes per annum (Evans, 2016).

Within this global context, the European Union (EU) produces 13.1 million tonnes of poultry meat (EUROPA, 2015) with Poland being the largest producer (15%) followed by France and then the UK (14% and 13%, respectively). Typical of developed nations, poultry producers in the EU have to comply with legislation that exceeds international standards and which increase the cost of production by an estimated 4.8 Eurocents per kg live-weight at farm level compared with many non-EU countries. Indeed, some EU countries choose to go even further than the EU standards and implement even more stringent national legislation partly to reflect public opinion about animal welfare and partly as a means of establishing a differential advantage in international markets.

In recent times the global market for poultry meat has been significantly impacted by growth in developing countries like China, Brazil, and India. However, the nature of the markets in developing countries is often somewhat different than in developed countries so, for example, consumers in

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developing countries often prefer dark meat cuts which are cheaper than white meat, so managing the carcass balance can be a challenge for organizations operating in both developed and developing countries. Also, in many developing countries there is much less legislation relating to matters such as environmental protection, animal welfare, and food safety, which means that the costs of compliance are much lower than in developed countries. As a result, production costs after slaughter are reported to be significantly lower in non-EU countries (Van Horne and Bondt, 2013) and EU import levies are used to protect companies producing within the EU although often with limited success.

The growth in the global market for poultry meat represents an excellent opportunity for producers but the different characteristics of markets in developed and developing countries reveals a complex situation that requires careful consideration if it is to be exploited efficiently and effectively. The aim of this chapter, therefore, is to identify and consider some of the key factors impacting on the growth and development of poultry meat markets in developed and developing countries. This will be carried out using a case-study approach, with the UK being used as an example of a developed country and China being used as an example of a developing country. The chapter will also make use of original primary research that has been conducted in each country.

13.2 CASE COUNTRY A: UNITED KINGDOM

13.2.1 An Introduction to the UK

The United Kingdom (UK) comprises England, Scotland, Wales, and Northern Ireland. It is subject to democratic governance from its capital city of London via a constitutional monarchy and a parliamentary system. The poet William Blake is credited with the phrase "a green and pleasant land" which is derived from his poem *Milton a Poem* (Cox, 2004) and understood to be a collocation for the landscape which, being located in North West Europe, receives prevailing winds from the South West that have been warmed by the Gulf Stream and which give the UK a temperate climate with plentiful rainfall and mild temperatures all year round.

According to the United Nations (2015), the UK has a population of 63 million people with a population growth rate between 2010 and 2015 of 0.6%. Some 12.4% of the population is made up of international migrants. In 2014 17.6% of the population was aged 0−14 while 21.7% of the male population and 25.0% of the female population was aged 60+ and 82.3% of people lived in an urban setting.

The United Nations (2015) also reported that in 2013 the Gross Domestic Product (GDP) of the UK was $2,678,455 million while GDP per capita was $42,423.40 and Gross National Income (GDI) per capita was $42,098.20. Exports accounted for some $548,041.9 million while imports in the same

period were valued at $657,222.5 million. Unemployment was relatively low at just 7.5% of the working population, and the government spent 6.0% of GDP on education.

The UK has a long, rich, and diverse cultural history extending back several thousands of years. Although English is the predominant language there are still areas where Welsh, Scottish Gaelic, and even Cornish are spoken. The Industrial Revolution started in Britain and so large areas of the country were heavily industrialized although that has changed somewhat in recent times as Britain has evolved into a postindustrialist society. The UK has produced many writers and poets of note including William Shakespeare, Geoffrey Chaucer, Wilfred Owen, and more recently Graham Greene and Roald Dahl. The country also has a strong connection to the popular music industry having spawned the likes of The Beatles, Elton John, Robbie Williams, and Adele. Religions such as Anglicanism and Catholicism have played an important part in the development of the UK, though today the population is largely secular. The most popular sport in the UK is football, although many other sports such as golf, cricket, rugby, and tennis are also played.

The agricultural sector in the UK is intensive and efficient, being characterized by a high level of mechanization and only 1.2% of the working population is employed in the sector (compared to 18.9% being employed in the industrial sector). The sector produces approximately 60% of the UK's food needs. Concentration and control is a feature of the agri-food supply chain in the UK where four main retailers dominate the marketplace though their market share is increasingly challenged by the rise of discounters such as Aldi and Lidl. The UK exports £18.8 billion of food which is mostly processed and imports food and drink to the value of £39.5 billion (Department of the Environment Food and Rural Affairs (DEFRA), 2015a) resulting in a trade deficit for food-related products.

13.2.2 The Poultry Industry in the UK

The economic impact of the British poultry industry's contribution to UK GDP is estimated to be £3.6 billion (British Poultry Council, 2015) and the sector supports 79,300 jobs in the UK. The poultry supply chain is highly integrated and consolidated with almost all broiler production units associated with just six organizations, over a third under the management of just two of these organizations (Crane and Laney, 2015), and 85% of turkey production also in the hands of just two organizations. Poultry premises stocked with more than 50 birds have to be registered and the list shows almost 800 such premises in England housing over 84 million birds and averaging over 100,000 birds per premise. Of these, 80% of broiler birds (65% of units) were reared in barn-based production systems, with 5% (21% of units) free-range, and 1% organic (6% of units).

Three quarters of the poultry consumed in the UK is reared on UK farms showing 77% self-sufficiency (Agricultural and Horticultural Development Board, 2015). Within the poultry category, chicken accounts for 92% of the poultry reared for meat (with turkeys 3%, ducks 2%, and geese 0.1% of production) providing just over 900 million chickens reared for slaughter in the UK for meat production (British Poultry Council, 2015). Although there has been a general increase in chicken sales since 2000 (18% on the year 2000 level), there has been some steadying of growth in the sector in recent years (Department of the Environment Food and Rural Affairs, 2015b) with the production of poultry meat stabilizing in 2014 at 1.65 million tonnes. Conversely, turkey meat production has shown the largest fall within the sector, falling 8.5% to 171,000 tonnes. Overall, the poultry meat sector is valued at £2.26 billion.

While the UK is a major international producer of poultry meat, providing 13% of the total EU production in 2014, it is not self-sufficient and has run a long-term trade deficit in poultry meat (although this narrowed in years 2006 and 2013) according to the UK Department of the Environment Food and Rural Affairs (2015b). Imports have increased steadily from 1997 to 423,000 tonnes in 2014 and exports have also risen from 258,000 tonnes in 2009 to 353,000 tonnes in 2014 with a value in real terms of £305 million. The Netherlands is the UK's main trade partner for poultry meat, accounting for 43% of imports in 2014 with Poland, Germany, and the Irish Republic combined accounting for a further 30%.

Similarly, the trade deficit for poultry meat products (which include prepared, preserved, salted, or cooked poultry meat) remains very wide with 306,000 tonnes of imports in 2014 (Department of the Environment Food and Rural Affairs, 2015b) compared to just 49,000 tonnes of exports. The main trade partners for poultry meat products are Thailand accounting for 41% of imports, Brazil 13%, and the Irish Republic and the Netherlands accounting for 10% each in 2014.

13.2.3 The Market for Poultry Meat in the UK

The consumption of poultry meat has steadily increased to 33.1 kg per capita per year in the UK (Agricultural and Horticultural Development Board, 2015) with poultry meat being the most popular meat eaten in the UK accounting for 47% of all meat purchased by weight. Chicken accounts for 91% of sales (British Poultry Council, 2015) with the majority of poultry purchased from the supermarket where own-label accounts for 67% of new launches of poultry products (Mintel, 2014). The growth of discount supermarkets including Aldi and Lidl has increased the focus on prices.

According to the DEFRA (2015b) the price and cost of food is a major concern for consumers in the UK with 36% of shoppers citing it as the most important factor in their purchase decision. Quality or performance of the

food was rated as the most important influence by 18% of respondents followed by taste or smell (13%) and health (10%). The influence of brand or ethical production (which includes organic, free range, and freedom food) were considered the least important factors. When looking specifically at poultry meat purchases, Mintel (2014) reported that low price and British origin (which may help elicit a feeling of trust), are commonly seen as the most important factors influencing consumer choice. These factors are more important than high animal welfare, particularly among the older generations.

In the UK there appears to be a disconnect between the understanding of consumers and farming practices relating to standard and higher welfare production, and understanding of the labeling for organic, free range, and Red Tractor assurance, which may stem from a generational divide in consumers' attitudes relating to the farming and production of poultry. Educating the public about the real differences in production may lead to better understanding and uptake of higher-welfare poultry (Mintel, 2016). However, there is some evidence to suggest that younger buyers may be swayed more by poultry products being quick to cook, promoted using emotional appeals, products having immediate benefits, ethnic and cultural diversity, and even a desire not to have to touch the meat. Indeed, while the differences in the generational divide may pose a challenge they may also provide opportunities for the poultry sector to innovate in packaging, promotion, added-value products, and products.

Poultry meat is regarded as a value-for-money purchase in the UK, with the average household spending £2.30 per week on fresh, chilled, or frozen poultry. Its popularity has been driven by its affordable price compared with red meat as well as having a versatile and healthy image. Indeed, the British Poultry Council (2015) report that the price of poultry meat increased by only 13% between 2008 (when the recent recession started) and 2014, which is significantly less than the 23% increase in food prices in general and also less than beef and lamb which increased in price by 35% and 42%, respectively, in the same period.

While the value of the processed frozen poultry market has declined (Mintel, 2014) and frozen poultry may be perceived as being of lesser quality, there may be opportunities for innovation in resealable packaging, or preportioned servings to freeze in the home (Pelligrini, 2014) at least in part as an attempt to reduce waste.

13.3 CASE COUNTRY B: CHINA

13.3.1 An Introduction to China

The People's Republic of China is a vast country covering 9.6 million square kilometers that stretches from the border with Russia in the North to

the sub-tropical island of Hainan in the South, and extends some 3000 miles across the eastern part of the Eurasian landmass. It is geographically and culturally diverse (Dillon, 2009) and Chinese society is regarded as a series of contradictions and complexities (Dillon, 2009; Mitter, 2008).

The United Nations (2015) reports that the population of China currently stands at approximately 1.39 billion people which makes it the most populous country in the world. However, the one child per family policy means that the population growth rate is fairly modest at just 0.6% while international migration is also limited with immigrants accounting for just 0.1% of the population. Some 18.1% of the population is aged from 0−14 with 15.2% of the female population and 13.8% of the male population being aged 60+. Although there has been significant economic migration toward the cities in recent times, the urban population still only accounts for 54.4% of the overall population which means that a significant proportion of the population live in rural areas.

Chinese civilization and culture can be traced back thousands of years (Dillon, 2009) and with discoveries and inventions including gunpowder, paper, and printing being credited to Chinese visionaries, China has been regarded as a world leader in science and technology. However, development in China was set back somewhat in 1949 by the rise to power of the Communist regime led by Mao Tse-tung who imposed a Soviet-style, centrally planned economy on the country and instigated a series of brutal purges in what became known as the cultural revolution. The net effect was to destroy much of the commercial apparatus of the country and leave many millions to die of starvation and it was not until Mao's death in 1976 that the situation was to change.

It was left to Deng Xiaoping, Mao's Presidential successor, to introduce a reform program that would transition the economy toward a mixed-market model. In the first instance, in addition to reducing state control over the lives of its citizens, the new government dismantled the system of agricultural collectivization put in place by Mao, disbanded the farming communes, reintroduced the concept of private land leases, and established Special Economic Zones (SEZs) where businesses would be subject to preferential commercial rates and taxes.

Successive presidents have continued to reform the economy to the present day and the current President Xi Jingping has introduced legislation to address slowing economic growth and in particular issues associated with weakening demand for Chinese goods and reduced exports, overextension in the domestic credit market, and severe environmental problems stemming from rapid economic growth. However, while the effect of the reforms has varied over time the Chinese economy has achieved a GDP growth rate of up to 11.2%, helped somewhat by China joining the World Trade Organization in 2001 and reentry into international trade, and they have been credited with improved living standards that have taken approximately 150 million people out of poverty.

Today, China is a one-party state subject to government by the Communist party from its capital city of Beijing. Government investment in scientific research and education remains significant with $163 billion spent on the former in 2012 and 1.9% of GDP spent on the latter between 2007 and 2013.

According to figures published by the United Nations (2015), China exported $2,209,007.3 million and imported $1,949,992.3 million worth of goods in 2013. This makes China the biggest exporter and the second largest import market in the world. Subsequently, Chinese GDP reached some $9,181,204 million in 2013 (United Nations, 2015) which makes the Chinese economy the second largest in the world even though GDP and gross national income per capita remain fairly low at $6,626.3 and $6,594.7, respectively (United Nations, 2015). In 2013 unemployment was low at 4.6% and, as might be expected, some 29.5% of the working population was employed in industry while 34% were employed in agriculture. Indeed, although reduced somewhat from the heady days of the 1990s and relying heavily on exports, China still has one of the fastest-growing economies in the world and low labor costs and relatively high productivity still make China a very attractive proposition for international manufacturing companies.

13.3.2 The Poultry Industry in China

According to Krystallis et al. (2012) the poultry industry in China has developed rapidly in recent times in response to changing market demand. In terms of volume, China became the world's largest producer in 2008 when production of poultry and eggs reached 27,017,000 tons (Ministry of Agriculture of the People's Republic of China, 2013). However, since this time production has risen still further and the National Bureau of Statistics (2014) reported that production of poultry meat alone reached 28.76 million tons in 2013 with much of this being destined for overseas markets. Indeed, the Ministry of Agriculture of the People's Republic of China (2013) reported that in 2011 China exported some 12,000 tons of poultry meat which was valued at about $31 million and imported 19,400 tons that was valued at $40 million.

The growth in production volume has, however, not been without some set-backs and in recent years, as with many Asian countries, the poultry industry in China has been subject to outbreaks of Avian Flu. For instance, in March 2013, an outbreak of H7N9 Avian Flu caused a huge decrease in poultry meat consumption and a concomitant fall in prices. Poultry production companies reduced their production capacity and many went broke. Indeed, according to research completed by the China Animal Agriculture Association (CAAA), during the first 6 months of the outbreak, farms lost a total of 600 billion Yuan. Further, in the first few months of 2014, H7N9

had spread throughout Zhe Jiang and Guang Dong provinces and had had a negative impact on more than 40 million farms.

In addition to a significant expansion of the industry, there have been major changes to the nature and make-up of the poultry supply chain in China, again due to developing market demand. Specifically, increased market demand for better preservation (especially cold storage) and packaging as well as a requirement for higher product quality led to changes in terms of breeding, processing, shipping, and marketing.

13.3.3 The Market for Poultry Meat in China

Poultry meat accounts for 19% of the meat market in China (Zhou et al., 2012; Wang, 2007). This may still be somewhat distant from pork which accounts for 65% of the meat market, but is nevertheless a substantial market in its own right, with chicken being the most popular type of poultry meat.

The National Bureau of Statistics (2014) confirms that it is those in the upper echelons of society that spend the most on poultry products (see Table 13.1) and so it seems reasonable to assume that it is these same people who eat the most poultry products too. However, as many of these people also reside in the cities this then leads to a significant difference between poultry consumption in urban areas and poultry consumption in the rural areas (see Table 13.2). It is also apparent that the market for poultry products is growing faster in urban areas and that while consumption in Beijing is far greater than in other Northern regions, much more poultry is

TABLE 13.1 Chinese Urban Household Poultry Product Purchases (Yuan/Person/Year)

	2010	2005	2000	1995
Total average consumption	914.22	564.91	411.31	416.27
Lowest income	563.93	343.43	283.41	286.72
Low income	728.82	433.51	340.08	349.33
Lower-middle income	835.87	514.28	388.58	382.18
Middle income	955.23	584.52	422.22	422.67
Upper-middle income	1051.41	655.83	453.29	462.95
High income	1142.37	716.12	485.99	496.70
Highest income	1242.16	756.41	534.10	550.08

Source: The National Bureau of Statistics of China, 2014. Poultry. Available online at: <http://data.stats.gov.cn/> (accessed 10.01.14).

TABLE 13.2 China Household Poultry Product Purchases (kg/Person/Year)

Year	Urban	Rural	Difference
2005	8.97	3.67	5.30
2006	8.34	3.51	4.83
2007	9.66	3.86	5.80
2008	8.00	4.36	3.64
2009	10.47	4.25	6.22
2010	10.21	4.17	6.04

Source: The National Bureau of Statistics of China, 2014. Poultry. Available online at: <http://data.stats.gov.cn/> (accessed 10.01.14).

consumed in the South East of China than in the Northern provinces (National Bureau of Statistics, 2011).

The outbreaks of Avian Flu within the domestic poultry production industry and other issues such as the feeding of hormones have impacted consumer confidence and the market. Indeed, Luo and Liu (2011) reported sharp fluctuations in the domestic price of poultry products as a consequence of such issues while Xinhua (2013) noted that following an outbreak of Avian Flu in 2013 the price of a 2.5-kg Broiler chicken dropped to just 0.24 Yuan.

In the Chinese context, Lu et al. (2013, p. 351) argue that "animal welfare and animal rights are difficult to understand, because these (are) foreign concepts (that) were introduced into mainland China only in the early 1990s" but with economic development, greater access to information, and a number of very-well-publicized animal cruelty cases "societal awareness of animal issues is rising very fast" in China (Lu et al., 2013, p. 351). However, the manner in which Chinese people engage with animals may appear ambivalent. According to Lu et al. (2013), large tracts of Chinese society have a long cultural legacy of compassion for animals based on Daoist or Buddhist beliefs while at the same time in some regions there is an equally long cultural legacy of eating dogs and other companion animals which is often linked to ethnic cultural beliefs. As a consequence, while people's views regarding animal welfare have changed radically in recent years and continue to develop rapidly "awareness of animal welfare is still inconsistent and rudimentary in China" (Lu et al., 2013, p. 355) and legislation is limited (Rao et al., 2014). Indeed, the poultry sector is no exception and You et al. (2014) report that while some two-thirds of the population have not actually heard of the term "animal welfare," 72.9% of the respondents to their survey were of the view that for reasons of food safety, rearing conditions of animals should be improved, 65.8% supported the establishment of new laws to

improve conditions for animals, and more than half would pay a premium for these improved conditions.

13.4 UK AND CHINESE CONSUMER ATTITUDES TO POULTRY MEAT

The information on which this paper is based is derived from work initiated in the 1990s by Jones and Parrott (1997) and updated regularly in the intervening years to date. The research instrument used to collect the data was developed from a review of the literature and focuses primarily on contemporary issues relating to in-home consumption of poultry meat. These include purchase patterns (Vukasovic, 2010; Sismanoglou and Tzimitra-Kalogianni, 2011), consumer perceptions (Charles, 1975; Yeung and Morris 2001; Kennedy et al., 2004; Vukasovic, 2009, 2010; Sismanoglou and Tzimitra-Kalogianni, 2011; Vukasovic, 2012), and the factors influencing consumer decision making (Kennedy et al., 2004; Vukasovic, 2010; Sismanoglou and Tzimitra-Kalogianni, 2011).

The UK fieldwork was conducted in 2013. A pilot study was conducted to ensure the validity of the research instrument before 3000 questionnaires were delivered by hand to a cross-section of housing types for self-completion by the respondents. A total of 348 questionnaires were returned completed which, according to West (1999), gives the UK data 90% confidence with a margin of error of 5.0%.

In China the fieldwork was completed in 2014. The first stage involved testing the validity of the questionnaire in the Chinese context via a pilot study. Once the validity of the questionnaire had been established, 500 questionnaires were distributed outside major supermarkets in five key shopping districts in Beijing (Haidian, Shijingshan, Xicheng, Chaoyang, and Fengtai). A total of 432 completed questionnaires were returned which gives the Chinese data 95% confidence with a margin of error of 5.0% (West, 1999).

The data was analyzed using SPSS 19 and the UK data was used as the basis for a refereed journal paper (Walley et al., 2014). Brief descriptive data relating to the UK and Chinese samples is presented in Tables 13.3 and 13.4. Although neither can be considered perfectly representative, both sample data sets extended across the key population dimensions of gender and age and both were considered to incorporate the main consumer groups.

13.4.1 Consumption and Purchase of Poultry Meat

Data concerning the proportion of the samples that eat poultry meat is presented in Table 13.5. With 94.0% of the UK sample and 96.5% of the Chinese sample eating poultry meat it is clear that poultry meat is consumed by the majority of respondents.

Data concerning the purchase frequency of poultry meat is presented in Table 13.6 and there are some apparent differences between the UK and

TABLE 13.3 UK Sample Data

	Population (000)		Sample	
Age (years)				
16–24	6285	14.7%	18	5.2%
25–29	3651	8.6%	20	5.7%
30–39	7058	16.6%	49	14.1%
40–49	7436	17.4%	61	17.5%
50–59	6397	15.0%	64	18.4%
60+	11,832	27.7%	136	39.1%
Gender (16+)				
Male	20,937	48.7%	110	29.0%
Female	22,052	51.3%	246	70.7%

Source: Office for National Statistics, 2015. Available online at: <http://webarchive.nationalarchives.gov.uk/20160105160709/http://www.ons.gov.uk/ons/index.html> (accessed 24.03.16).

TABLE 13.4 China Sample Data

	Population (000)		Sample	
Age (years)				
16–24	166,121	17.8%	101	23.4%
25–29	93,136	10.0%	76	17.6%
30–39	167,011	17.9%	85	19.7%
40–49	201,900	21.6%	92	21.3%
50–59	140,252	15.0%	51	11.8%
60+	166,517	17.8%	27	6.3%
Gender (16+)				
Male	474,124	50.7%	138	31.9%
Female	460,809	49.3%	294	68.4%

Source: National Bureau of Statistics of China, 2013. 2013 Statistical Data. Available online at: <http://www.stats.gov.cn/enGliSH/Statisticaldata/AnnualData/> (accessed 29.04.16).

China. Whereas some 65.3% of the UK respondents purchase poultry meat either once a week or several times a week, only 41.2% of the Chinese respondents do likewise. Conversely, while only 7.7% of the UK respondents purchase poultry meat either 2–3 times a month or once a month, 38.4% of the

TABLE 13.5 Consumption of Poultry Meat

	UK (%)	China (%)
Eat poultry meat	94.0	96.5
Do not eat poultry meat	5.5	3.5

TABLE 13.6 Frequency of Purchase

	UK (%)	China (%)
Several times a week	21.0	10.6
Once a week	44.3	30.6
2–3 times a month	20.7	20.6
Once a month	6.6	17.8
Once every 2–3 months	1.1	6.5
Rarely	0.6	9.5
Never	5.7	4.4

TABLE 13.7 Type of Poultry Meat Purchased

	UK (%)	China (%)
Chicken	94.3	70.8
Turkey	41.7	1.4
Duck	17.5	24.8
Guinea fowl	1.4	0.0
Quail	0.0	1.3
Goose	0.0	1.3

Chinese respondents buy this frequently. It appears, therefore, that poultry meat is a much more frequent purchase in the UK than it is in China where, for many, it remains a relatively infrequent purchase. This could be because poultry meat is a relatively affordable meat in the UK where it has also been promoted as being relatively healthy or because it is a relatively expensive meat in China where it is still regarded as an occasional luxury purchase.

Data concerning the type of poultry meat purchased is presented in Table 13.7. It is apparent that the three most popular types of poultry meat

in the UK and China are chicken, turkey, and duck. However, the proportion of respondents purchasing the different types of poultry meat does vary between the countries. While chicken is the most purchased poultry meat in both countries, 94.3% of UK respondents purchase it while only 70.8% of the Chinese respondents buy it. Similarly, while 41.7% of UK respondents buy turkey only 1.4% of Chinese respondents do and conversely, while 17.5% of UK respondents buy duck some 24.8% of Chinese respondents do. These differences could be explained to some extent by variations in relative price but it seems just as reasonable to assume that they are explained by product availability and cultural differences between UK and Chinese consumers.

Data concerning the place where respondents purchase poultry meat is presented in Table 13.8. As might be expected, the proportion of respondents purchasing poultry meat in the different outlets does vary between the UK and China. While the majority of both the UK and Chinese respondents purchase poultry meat from supermarkets, a slightly higher proportion of UK respondents do so (80.7%) than Chinese respondents (74.0%). However, a much higher proportion of UK respondents (26.4%) purchase poultry meat from Butcher than Chinese respondents (4.0%), while the converse is true of markets, where 18.0% of Chinese respondents buy poultry meat but only 8.6% of UK respondents. These differences may very well be explained by the historical and cultural differences between the two countries and the fact that a market-based system of exchange has been in existence for a lot longer in the UK than in China, which has permitted better development and refinement of the distribution system. Indeed, these same factors may account for 8.3% of the UK respondents purchasing poultry meat from farm shops as opposed to no Chinese consumers frequenting this form of outlet, which suggests that it does not (yet) exist in China.

The data presented in this section indicates that UK and Chinese consumers demonstrate different consumer behavior in the purchase of poultry meat with the former buying more frequently and tending to buy chicken

TABLE 13.8 Place of Purchase of Poultry Meat

	UK (%)	China (%)
Supermarket	80.7	74.0
Butcher	26.4	4.0
Market	8.6	18.0
Farm shop	8.3	0.0
Local grocer	2.0	2.0
Online	1.4	2.0

and turkey, while the Chinese consumers buy less frequently and focus on buying chicken and duck. It is also apparent that while the majority of UK and Chinese consumers buy poultry meat from supermarkets, in the UK butchers, markets, and farm shops are also important retail outlets, while in China the only other retail outlet of any significance is markets. It is important, therefore, that poultry meat suppliers acknowledge the differences in UK and Chinese consumer behavior, particularly with regard to retail outlet frequented, in order that they may exploit the respective markets successfully.

13.4.2 Purchase Factors

Data concerning the importance of factors impacting the purchase of poultry meat is presented in Table 13.9. Chinese data relating to country of origin and welfare assurance is omitted because the Chinese research team felt that these matters would have very little meaning for the Chinese consumer, and so the questions about their respective importance were omitted from the Chinese survey. However, these subjects were the focus of some of the Likert scale statements in a later section of the questionnaire and the data provides a fascinating insight into Chinese consumer perceptions of country of origin and welfare assurance (see Table 13.10 and the accompanying narrative).

TABLE 13.9 Mean Scores of Factors Impacting the Purchase of Poultry Meat

	UK	China
Use-by date	4.30	3.76
Taste	4.18	3.28
Country of origin	3.84	–
Welfare assurance	3.76	–
Price	3.68	2.74
Quality standard	3.65	3.84
Fat content	3.57	2.69
Weight	3.04	2.42
Packaging	2.56	2.57
Brand name	2.45	3.07

1 = not important, 5 = very important, 6 = don't know (omitted from mean calculation).

TABLE 13.10 Perceptions of Poultry

		UK	China
1	I am careful to cook poultry meat well to avoid ill health.	1.38	–
2	I disapprove of poultry being kept inside all the time.	1.54	–
3	Free range poultry meat is tastier than intensively reared meat.	1.85	1.85
4	Poultry produced in Britain/China is the best.	1.87	2.12
5	I am concerned about antibiotic residues in poultry meat.	2.16	1.69
6	I am prepared to pay more for meat with welfare assurance.	2.30	2.64
7	I consider the quality of poultry meat in restaurant meals.	2.36	–
8	I do think about recent health scares when I buy meat.	2.37	–
9	Organic poultry meat tastes better than other poultry meat.	2.38	2.31
10	My buying behavior has changed in light of adverse publicity regarding poultry production.	2.49	1.83
11	I look on the meat label for welfare guarantees.	2.50	–
12	The risk of consuming genetically modified (GM) material that has been fed to poultry is low.	2.58	–
13	I frequently eat poultry when I "eat out."	2.65	2.35
14	I rarely consider the quality of poultry in ready-prepared meals.	2.87	3.00

1 = strongly agree; 2 = slightly agree; 3 = neither agree nor disagree; 4 = slightly disagree; 5 = strongly disagree; 6 = don't know (omitted from mean calculation).

In the UK, the "use-by date" is the most important factor and brand name the least important factor impacting on the purchase of poultry meat; however, it is evident that Chinese consumers prioritize the factors differently. Indeed, in China quality standard is the most important factor and weight is the least important factor impacting on the purchase of poultry meat. Again these findings appear intuitively logical as in the UK the consumers see the "use-by date" as a key indicator of product quality and freshness while brand name has less meaning because the majority of poultry meat is not branded beyond carrying the label of the retailer from where it is bought and many UK consumers would not necessarily understand the retailer's name to be a brand name. Similarly in China, consumers value quality standards so highly because they would see it as the primary indicator of product quality while weight would be of much lower importance because most poultry meat would not be prepacked and so just the required weight would be purchased.

The most important point arising from this data is that UK and Chinese consumers have a different set of priorities when it comes to purchasing poultry meat and that if advertising and marketing is to be successful it needs to reflect this and use different messages and different creative forms in the two different countries.

Data concerning the perceptions of consumers (and purchasers) of poultry meat is presented in Table 13.10. The statements that are the basis of Table 13.10 elicit information in the form of perceptions and opinions about poultry, poultry meat, and poultry marketing in their own right but also serve to provide insight into the factors shaping buying behavior. General agreement with the statements "I consider the quality of poultry meat in restaurant meals," "I frequently eat poultry when I 'eat out'," and ambivalence toward the statement "I rarely consider the quality of poultry in ready-prepared meals" suggests that consumers do consider issues relating to the quality of poultry meat even when someone else prepares, or partly prepares, the food.

The statements are ordered according to how strongly the UK consumers agree with the statements and it is clear immediately that the data originating from China does not follow a similar ranking. So while UK consumers most strongly agree with the statement "I am careful to cook poultry meat well to avoid ill health," the Chinese researchers did not consider it worthwhile even asking this question (because it is not seen as an issue in China) and instead Chinese consumers most strongly agreed with the statement "I am concerned about antibiotic residues in poultry meat." Collectively, therefore, this would appear to confirm the point that the consumer agenda regarding poultry meat is different in China than in the UK and that organizations operating, or wishing to operate in China, must be sensitive to the wants and needs of the Chinese market.

13.4.2.1 Health

In terms of the issues underpinning the statements in Table 13.10, it is clear that in ranking the statement "I am careful to cook poultry meat well to avoid ill health" as that which they agree with most strongly, UK consumers are revealing that health is one of, if not the most important factor underpinning their buying behaviors. However, the fact that they agree somewhat less strongly with the other health-related statements ("I am concerned about antibiotic residues in poultry meat," "I do think about recent health scares when I buy meat," and "the risk of consuming genetically modified (GM) material that has been fed to poultry is low") suggests that UK consumer concern is currently linked more closely with cooking than with production techniques and health scares. It is also interesting to note that while the Chinese researchers did not include the health-related statements ("I am careful to cook poultry meat well to avoid ill health" and "I do think about recent health scares when I buy meat") because these matters are not seen as issues

in China, they did include the statement "I am concerned about antibiotic residues in poultry meat" which the Chinese consumers then agreed with more strongly than with any other statement. It is also interesting to note that in omitting the statement "the risk of consuming genetically modified (GM) material that has been fed to poultry is low," the Chinese researchers were acknowledging the Chinese consumers' acceptance of GM and that it is now a staple ingredient of many foods sold in China, something that is very different than in the UK. In summary, therefore, it would appear that health is a very important factor underpinning purchase decisions in both the UK and China but that the nature of the underlying issues is different in the two countries.

13.4.2.2 Production Methods

By agreeing strongly with the statements "I disapprove of poultry being kept inside all the time" and "free range poultry meat is tastier than intensively reared meat," UK consumers appear to reveal a concern for poultry production methods which in itself may be an important factor in the purchase decision. However, just as with health, consumer interest in poultry production methods is not simple as it originates from a number of underlying concerns. UK consumer agreement with the statements "I disapprove of poultry being kept inside all the time" and "I look on the meat label for welfare guarantees" appears to suggest concern for animal welfare while agreement with the statements "free range poultry meat is tastier than intensively reared meat" and "organic poultry meat tastes better than other poultry meat" seems to indicate a concern for product taste.

While the concern for product taste expressed by both UK and Chinese consumers might be expected and the apparent concern for welfare expressed by the UK consumers is not unexpected it is the fact that the Chinese researchers, again, omit the statements relating to welfare ("I disapprove of poultry being kept inside all the time" and "I look on the meat label for welfare guarantees") that is of much more interest.

Consumers in the UK and China are subject to a barrage of adverts on the TV and via other media as well as product placement within popular films and TV programs with much marketing making use of animals to convey their messages. In the UK, this has not only educated the populace about animals, but it has served to make people aware of the welfare issues surrounding human engagement with animals and even to ascribe human thoughts and emotions to animals. This latter phenomenon is known as "anthropomorphism" and is a concept that has been the focus of much research effort in the West (e.g., Geerdts, 2016; Miles and Ibrahim, 2013). Whether it is because UK culture makes UK consumers more susceptible to anthropomorphism or because they have been subject to the barrage of anthropomorphic advertising for longer, it appears that animal welfare is an

issue that makes concern for poultry production much more important in the UK than in China. It remains to be seen, therefore, whether Chinese culture which historically is very much orientated toward nature but which is changing fast, will reduce or negate the impact of anthropomorphism with respect to poultry production in China, or whether after a sufficient length of time the advertising barrage does create anthropomorphic tendencies in Chinese consumers such that welfare does become an issue underpinning purchase decision for poultry meat in China.

While it is not yet possible to confirm which way the Chinese market will go with this issue the fact that the Chinese consumers agree so strongly with the statement "my buying behavior has changed in light of adverse publicity regarding poultry production" may very well be an early indication that the Chinese market is evolving in much the same way in which the UK, and indeed many Western markets have done. Fortunately, the Chinese consumers just as much as the UK consumers also agree with the statement "I am prepared to pay more for meat with welfare assurance," and so it would appear that to some extent they will be prepared to pay for a higher level of poultry welfare.

13.4.2.3 Country of Origin

Country of origin is a factor impacting on the purchase decision for many products and services (Diamantopoulos et al., 2011) and because of this, Han (2010) suggested that it had received a considerable amount of research attention since the term was first coined in the 1960s. The person who first used the term was Robert Schooler (Schooler, 1965) who noted a tendency for people to favor products originating from their own nation state; a phenomenon that would eventually be referred to as "ethnocentricity." While the term "country of origin" was first used in respect of the national origin of products it is now used to refer to other aspects of sourcing such as country of assembly (Biswas et al., 2011) and country of brand (Uddin et al., 2013). Also, while much of the early research work focused on developed countries Hamin and Elliott (2006) note that now there is a growing body of knowledge relating to country of origin and developing countries.

Indeed, the fact that both the British and Chinese respondents to this study indicate strong agreement with the statement that poultry originating from their own country is the best suggests that ethnocentricity impacts on the populations in both countries and that country of origin does have a significant impact on people's purchase decisions in both countries. However, the fact that the Chinese researchers did not consider country of origin sufficiently important to include it for assessment as a purchase factor may mean that in China it is unrecognized and is either an unexploited or underexploited marketing asset. Overseas producers, however, should also remember that the ethnocentric tendency to favor poultry produced in the home country may imply the need for local production.

13.5 CONCLUSION

A complex and dynamic macro-environment means that international trade is a significant challenge for many organizations but one of the most important contributory factors is the differences between markets and consumers in different countries. This chapter is based on studies of markets and consumers in the UK and China which have been chosen to represent typical developed and developing countries and to highlight the often complex interactions between consumer's attitudes, opinions, and perceptions that shape consumer behavior. Indeed, a number of key points arose from this study that might inform organizations either involved in or contemplating international trade involving poultry meat.

A first point arising from this study is that developing countries may well represent very attractive strategic opportunities for companies involved in poultry-meat production. Not only are markets such as that found in China large and growing rapidly, but the quality of products produced in developed countries is often attractive to consumers who are not always confident in products produced domestically, and who may be willing and able to pay a premium for products produced overseas.

A second point, however, is that while an apparent opportunity markets in developing countries are not necessarily easy to understand and exploit. From this study it is apparent that while the Chinese market values some aspects of the purchase proposition of poultry meat in the same way that consumers in the UK do, for instance quality and use-by dates, it also demonstrates aspects that are different, such as the preference for different parts of the bird. These differences may be explained by such things as the historical development of the market and differences in culture, but they do serve to remind us that a proposition developed in one country may not necessarily work in another. Such propositions extend to include advertising that focuses on a different set of priorities as well as different poultry and cuts. It really is necessary to undertake research in foreign countries in order to gain the level of understanding that might permit success.

Another point is that while some consumers in developing countries may perceive products produced in a developed country to be better than products produced domestically, that does not mean that they will buy them. The research conducted in China reveals a fairly strong tendency for consumers to be ethnocentric, which means that they may well prefer to buy poultry meat that originates in China. The obvious implication here being that a poultry producer wishing to serve the Chinese market may well have to consider producing in-country rather than producing poultry meat in a different country and simply importing it.

A fourth point is that while it is easy to equate markets to countries, a country as big as China may actually comprise a number of separate and distinct markets. For instance, You et al. (2014, p. 5) note that because

economic and social development varies across China then "people from different regions tend to take different attitudes on the same subject." This means that poultry companies considering entering the Chinese market or any other large overseas market must recognize the potential for diversity and not automatically assume that they are serving just one big market.

Finally, it is important to recognize that survey research like that which underpins this chapter, while providing a useful insight into the consumer psyche, is actually a snapshot in time, but that consumers' attitudes and perceptions are dynamic and over time are almost certainly going to develop and change. The key, therefore, to a poultry-producing organization successfully exploiting an overseas market is not just about understanding the market as it stands at present but also using data such as that presented in this chapter and extrapolating forward to achieve a good understanding of what is likely to happen in the future too.

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Chapter 14

Animal Welfare and Poultry Meat in Alternative Production Systems (and Ethics of Poultry Meat Production)

Cesare Castellini and Alessandro Dal Bosco
University of Perugia, Perugia, Italy

14.1 INTRODUCTION

An important topic in the poultry production is the use of less stressful farming techniques more respectful of animal welfare, environmental impact, and nutritional value of meat. Such issues are differently interpreted by the actors in the poultry chain: producers outline animal welfare mainly in terms of good health, feeding, and housing, and consider the aspects related to the ability to express appropriate bird behavior as less important (Tuyttens et al., 2014). This belief contradicts the perception of the consumer and the assumptions of the European Welfare Consortium which affirms that the intensive meat production obtained with fast-growing chicken strains "is not accompanied by a suitable level of animal welfare and health" and should be better addressed.

Indeed, the equilibrium between productive performance and animal welfare is not easily reachable in intensive poultry systems which are based on high bird density, large number of animals per farm, and on birds selected for high growth rate, feed conversion, and high proportion of breast muscles.

Theoretically, the alternative poultry production system (organic, free-range) is founded on a different approach, keeping under consideration the main pillars of sustainability, animal welfare, and food quality. To reach this goal, the definition of guidelines aimed to maximize the genotype/environment interaction is crucial.

On the basis of these considerations, the main critical aspects of alternative poultry systems are related to the choice of the best genotype, housing, and management system (pasture, feeding plan, range enrichment, etc.). Furthermore, up- and downstream infrastructure (slaughter houses, hatcheries

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with adapted genetic strains), services (knowhow, phytotherapeutic drugs, vaccines for small number of birds, etc.) will require development.

14.2 POULTRY GENETICS FOR ALTERNATIVE PRODUCTION SYSTEMS (WELFARE AND ADAPTATION)

The welfare of broiler chickens is enhanced by the access to free-range areas but the genetic strain strongly modifies the extent of such effects and the best results depend on the choice of genotypes able to utilize pasture. Producers are encouraged to choose birds selected for their ability to cope with the natural environment, well developed immune system, a low occurrence of injurious behaviors, good conformation and skeletal development, and an adequate (not too fast) growth rate.

Fast-growing birds are selected for daily gain and percentage of breast muscles, and are created for intensive poultry farms. These birds reach the market weight within 6−7 weeks and are tailored for living in extremely regulated conditions (both in terms of environment and veterinary control) and eat diets very concentrated in terms of digestible energy and protein.

Free-range and organic poultry systems require animals that are more resistant to fluctuations of environmental conditions thanks to a better immune resistance and adaptation to poorer diets and environments. Slow-growing genotypes are more adapted to these systems and birds take a longer period to grow (about 9−12 weeks).

Reviews on the welfare of organic poultry obtain similar conclusions: fast-growing chickens have a better growth rate and feed-conversion efficiency, combined with a low kinetic activity, low foraging behavior, lameness, and body lesions, indicating a poor adaptation to organic production (Zeltner and Maurer, 2009; Sossidou et al., 2012, 2015; Castellini et al., 2016a).

Lewis et al. (1997) observe that genetic selection for a faster growth rate modifies the behavior of birds, reducing the more energy-expensive activities (kinetic and foraging activity, reproduction, immune response). Weeks et al. (1994) showed that fast-growing broilers reared in free-range conditions make little use of pasture and tend to stay indoors and near the house, rather than foraging in the pasture. Accordingly, fast-growing strains should be avoided in alternative production systems.

Slow-growing strains, in comparison with fast-growing, show more active behavior, fewer heart and muscle abnormalities, less tendon degeneration (Bokkers and Koene, 2003), lower mortality, lower culling rate (Castellini et al., 2002a), and ascites. Slow-growing birds spend more time outdoors than indoors, whereas fast-growing strains have higher static activities, strongly associated with energy conservation, growth, and muscle accretion. Castellini et al. (2002b,c) confirmed that slow-growing chickens, if compared to fast-growing, have more intense walking activity, foraging aptitude, and in vivo antioxidant capacity.

The higher kinetic activity of slow-growing is also confirmed using Global Positioning System (GPS; Dal Bosco et al., 2010) in order to verify in an objective manner the kinetic activity of chickens. GPS gives information concerning the date, hour, environmental conditions, and coordinates of monitored birds and has been employed on male birds from two strains (slow- and fast-growing) from 73 to 80 days of age. GPS output shows that slow-growing chickens, reared free-range, cover an average daily distance of 1230 m, while fast-growing ones only cover 220 m (Fig. 14.1).

This higher kinetic activity also affects the muscle fiber characteristics and its enzyme activity: slow-growing chickens have a certain amount of αR fiber in breast muscle and an increased cross-sectional area of the *Ileotibialis lateralis* muscle, which together with behavioral data suggest that these birds are more adapted to extensive rearing systems (Branciari et al., 2009).

Typically, there are two categories of slow-growing strains: autochthonous breeds and commercial strains. Unfortunately, most of the autochthonous breeds, which have higher biodiversity and adaptability to extensive system, also show very low productive performance. Due to these limitations, they are not generally used as pure breeds and would require appropriate genetic (crossbred) and marketing strategies for their improvement and preservation (Dal Bosco et al., 2011).

Nonetheless, the large body of literature on the importance of slow-growing breeds and pasture for the correct design of organic system, the compulsory rules of organic legislation (EC 1999, 2007) and the National Agencies of control underestimated the role of these factors and permit (even if not recommended) the use of fast-growing birds (Sossidou et al., 2011). Avoiding fast-growing breeds should therefore be a priority for organic rules seriously committed for improving animal welfare and meat quality.

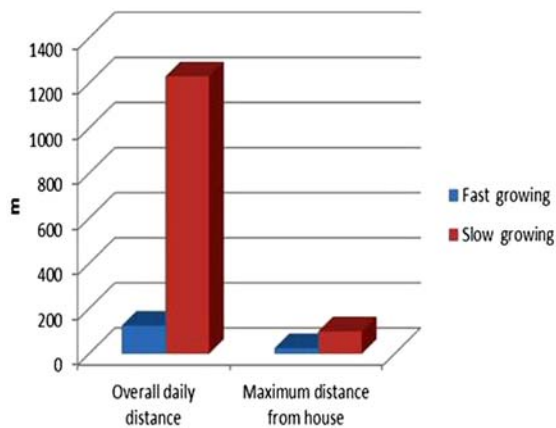


FIGURE 14.1 Global Positioning System outcomes of organic chickens. *Modified by Dal Bosco, A., Mugnai, C., Sirri, F., Zamparini, C., Castellini, C., 2010. Assessment of a global positioning system to evaluate activities of organic chickens at pasture. J. Appl. Poult. Res. 19, 213–218.*

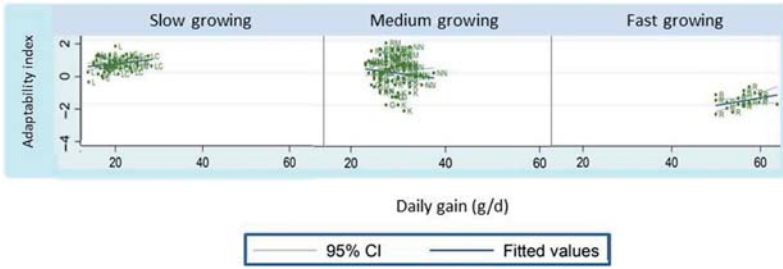


FIGURE 14.2 Fitted values of adaptability score versus daily gain within sub-groups (slow, medium and fast growing). *A*, Ancona; *CL*, crossbreed Cornish × Leghorn; *G*, Gaina; *K*, Kabir; *L*, Leghorn; *NN*, Naked Neck; *RM*, Robusta Maculata; *R*, Ross. *Modified from Castellini, C, Mugnai, C., Moscati, L., Mattioli, S., Guarino Amato, M., Cartoni Mancinelli, A., et al., 2016a. Adaptation to organic rearing system of eight different chicken genotypes: behaviour, welfare and performance. Italian J. Anim. Sci. 15 (1), 37–46.*

Thus, the main genetic prerequisite for the adaptation of chicken to an organic system is a moderate daily weight gain (less than 45–50 g/day), but different slow-growing strains could have a different adaptation to free-range. Castellini et al. (2016a) show that different genotypes with very slow, slow, and fast growth rates have different adaptations (Fig. 14.2). The adaptability index, estimated through 49 physiological, ethological, and productive endpoints, is the best in very-slow-growing strains and the worst in the fast-growing strains. Nevertheless, within the same sub-group, the correlation between daily gain and adaptation is not significant.

In conclusion, a moderate growth is a prerequisite for the adaptation to a free-range system, but there is still a lot of room for selecting poultry strains more adapted to alternative farming systems.

14.3 MANAGEMENT OF POULTRY PASTURE

As affirmed, the choice of animals adapted to the extensive rearing systems is the main condition for a wide use of the pasture (Sossidou et al., 2015), but also the management of the pasture is essential to ensuring its optimal use by birds.

The daily access to pasture increases the foraging behavior of birds and an early access facilitates the use of the outdoor range area. Young chicks easily learn how to forage grass and live protein sources, such as insects, worms, and grubs (Sossidou et al., 2011) and should be provided with outdoor access when the environmental conditions are proper and their feather cover (at around 3–4 weeks of age) enable them to thermoregulate.

To encourage the use of range, farmers should provide shade and shelter (shrubs, trees) throughout the outdoor area. Dal Bosco et al. (2014) report that chickens reared under olive trees use the run better and have a higher

herbage intake with respect to other birds without range enrichment. These birds explore and eat the pasture up to almost 50 m from the poultry house.

The use of pasture is also affected by the environmental temperature: few birds are ranging on cold winter days, but even in hot summer the birds ranging outside during daylight accounted for less than 15% of the total flock. Temperature and sun exposure have great impact on chicken foraging behavior. Chisholm et al. (2003) show that broilers spend more time foraging during the early morning (45% of birds) and late afternoon (29%), compared to 24% in the middle of the day.

14.3.1 Pasture Rotation

A proper pasture management should be performed for reducing the risk of soil erosion, weakening of the grass cover and pathogens infestation. Pasture should be rotated at least every cycle (2−3 months) and birds (and/or movable house) should be moved to a new location. Pasture rotation can be regulated by taking into consideration the lifecycle of the parasites, the soil type, the plant growth, and the bird number.

Site selection is another important aspect that should not be overlooked. Pastures used for poultry production should be well drained. Birds can drown easily or suffer from hypothermia when puddles of water form. Heavy, poorly drained soils are therefore generally unsuitable for pastured poultry (Gordon and Charles, 2002).

The outdoor stocking densities and the group size should allow enough grass for birds and should be balanced with soil type, the productivity of the land, the environmental impact, and health and welfare of the birds. In standard Mediterranean conditions, the minimum area for each bird (4 m^2) is generally not sufficient to have enough grass for the entire productive cycle and about the double (8−10 m^2/bird) is required (Sossidou et al., 2015).

14.3.2 Control Disease Outbreaks and Parasites

Even if it is not the focus of the present chapter, the maintaining of high health and welfare status with low usage of veterinary medicines is another focus of organic rearing systems. The EC regulations for organic farming state that organic livestock should be treated preferably with phytotherapy. However, almost no phytotherapeutic products are registered for livestock, and information regarding veterinary phytotherapy is really scarce (Mayer et al., 2014). It should be underlined that a healthy system does not automatically mean good health and welfare for the individual, and that extensive production systems expose poultry to a different pattern of disease challenge.

Anyway, in alternative systems all the preventative measures to control disease problems should be encouraged as pasture rotation, sanitation, and isolation of birds. The “all-in, all-out” approach is a useful way to maintain

flock health in a confined pen. Under this procedure, each batch of birds is treated as a unit from arrival on the farm until departure. Facilities are thoroughly cleaned and disinfected between batches of birds.

Also, producers should monitor their flocks by fecal samples and by observing the birds directly to monitor which internal and external parasites, respectively, exist on their farm.

14.3.3 Prevention of the Risk of Predation

Free-range-reared chickens are attractive to predators. Foxes are the most frequent predators, but other wild animals can cause damage and often kill or injure large numbers of birds. In addition to direct attacks, the presence of these predators can cause panic and losses through smothering and trigger outbreaks of aggressive behavior. The poultry unit site can influence the likelihood of such problems: thick plant cover close to the range area will encourage wild animals.

Permanent fencing can be expensive but should be substantial enough to exclude predators from the pasture of birds. Electrified fencing will generally provide satisfactory levels of protection against most predators. The fencing and energizer unit have to be well maintained in order to continue to work effectively. Grass underneath the fence must be kept cut to prevent shorting, and regular checks should be made on the connections between the fence and the transformer. However, the closing of poultry houses during the night is a suitable further protection against the intrusion of predators.

Chickens could also predated by raptors and the presence of trees could help them to feel more secure and more sheltered from sun and other environmental elements, so they can range further away from the houses and eat more forage. Dal Bosco et al. (2014), studying the effect of environmental enrichment (trees or tall grass stand) on the performance and herbage intake of free-range chicken observed a lower mortality due to predation in the presence of olive trees.

Multispecies grazing with ruminants can also aid in protecting poultry from predators that respect large animals.

14.3.4 Intake of Pasture and (Alternative) Poultry Feed

To ensure a suitable assimilation of grass nutrient by birds, the forage should be kept young. Older plants are less digestible than young leafy plants. Chickens ignore vegetation over about 10 cm high. In tall grass, they tend to make tunnels to feeders; tall grass holds moisture that can wet the feathers and the moisture may be tracked into the house. Poultry enclosures should be constructed so it is easy to eventually use a tractor to mow, plow, and plant. Ruminant grazing can help manage forage for poultry and avoid the need to mow or hay.

Comparative analysis of the crop content shows different feeding behaviors among different poultry strains. Lorenz et al. (2013) find that slow-growing broilers have higher feed intake in the pasture than fast-growing broilers, and that the pasture may represent between 10% and 15% of the total feed intake. Castellini et al. (2002b) confirm that the crop content of slow-growing, compared to fast-growing broilers, has less protein and energy and higher amounts of α-tocopherol and carotenoids, indicating higher grass ingestion.

Such differences in foraging behavior agree with the statement of Appleby and Hughes (1991), who display that free-range conditions allow poultry, mainly from slow-growing strains, to express species-specific behavior and their innate ability to provide for their own nutritional requirements. As an overall mean, the pasture intake may replace 5%−20% of a grain-based diet but, the percentage of substitution by these sources also depends on breed, age, environment, and quality of the forage, rendering it difficult to know which nutrients should be supplied and in which quantity. Birds raised on pasture still require a grain-based ration formulated to sustain their growth stage. In cases of very high grass intake, the fiber content of pasture may limit the nutrient utilization of the diet and the feed efficiency.

Dal Bosco et al. (2016) showed that bioactive compounds (vitamins, minerals, and polyunsaturated fatty acids (PUFAs)) are transferred from pasture to the meat of organic chickens and affect the oxidative processes of the meat. Slow-growing birds reared outdoors show a better antioxidant status and lower thiobarbituric acid reactive substances (TBARs) of blood plasma. The meat of birds also has higher levels of antioxidants and n−3 series PUFAs; nevertheless, the oxidative stability of drumstick muscle is lower, probably due to high kinetic activity and the higher peroxidability of meat (>PUFA, see Section 14.7).

Accordingly, the pasture can reduce the need for supplemental vitamins in poultry diet. Birds also consume roots, stems, and invertebrates. Coletta et al. (2012), comparing the ratios of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), show that differences in the diets (corn−soybean based, milled-corn, and free-range) reflect in the stable isotope composition of poultry tissues. Authors attribute the difference between the free-range and the barn-raised chickens (the significantly higher $\delta^{15}N$ of free-range) to major ingestion of animal proteins such as earthworms and insects.

The use of earthworms and insects as poultry feed is not new (Hilton, 1983) and some Authors suggest the possible use of alternative protein feedstuffs such as earthworm meal. The earthworm has an equal or higher efficiency than other proteic meals due to the high presence of essential amino acids. Khusro et al. (2012) reviewed that a huge number of insects (silkworms, locusts, fly larvae, crickets, and grasshoppers) can be fed to chickens without compromising their performance and meat quality.

Feeding insects to poultry could be beneficial in developing alternative formulation to overcome, for example, the use of genetically modified feed

(soybean) that is avoided in organic feed. Anyway, although EFSA gave a preliminary position on the use of insect in feed and food (EFSA, 2015), the safety and the economic viability of feeding insects to poultry needs to be further assessed.

Future researches should investigate these issues in more detail both by establishing production models which include insects or other less environmentally impacting protein sources in the poultry diet (see Section 14.5).

Moreover, it should be considered that the dietary recommendations of slow-growing strains, due to the lower growth rate, are generally 20% lower in terms of protein and energy (NRC, 1994) by making the feed formulation much more easy and sustainable (Perella et al., 2009; Dal Bosco et al., 2013).

14.4 HOUSING SYSTEMS (MOVABLE OR STATIONARY)

The management of free-range birds is very complex due to the uncontrolled environmental conditions. The spread of disease could be higher for outdoor-raised birds during wet and rainy conditions and when mosquitoes are active. Standing water as well as runoff water from heavy rains can be health hazards for chickens on pasture.

Even if the use of outdoor areas should be encouraged in different ways (see Section 14.3), a comfortable poultry house must always be available. The indoor density depends on several aspects and could be higher (16 birds/m^2) than stationary houses when the flock does not exceed 500 birds and the house is mainly used by birds during the night. This high indoor density requires the use of perches and the presence of good litter.

Transportable units should be moved frequently to decrease the risk of parasite or disease transmission, and to ensure the land does not become poached or polluted. Poultry producers with stationary systems are encouraged to create a buffer system between the stationary barn and the outdoor paddock. Buffer zones can take several forms from covered perches to concrete areas that can be cleaned. These areas help reduce nutrient loading in the area directly around the house as well as help to keep excess dirt from soiling the indoor facilities.

In any case, the house material should be easily cleanable and disinfectable.

14.5 SUSTAINABILITY OF PASTURE-BASED POULTRY REARING SYSTEMS

The term “sustainability” refers to an integrated system of plant and animal production that contributes to satisfying human food needs, to enhance environmental quality by making the most efficient use of natural resources and to sustain the socioeconomic conditions of farmers.

The need for environmental sustainable food production has never been greater than today. About one-third of arable land is used for producing feed for animals and this proportion is expected to increase further rendering the pressure of animal production on natural resources unsustainable (Alexandratos and Bruinsma, 2012). Huge amount of cereals eaten annually by livestock (about 70%) could feed about 3.5 billion humans and feeding animals human food causes big conversion losses (from 4- to about 20-fold according to the animal species; Wilkinson, 2012). Assuming that animal protein remains a relevant part of human consumption, we must figure out how to keep livestock in favorable ways for individuals, communities, and the globe.

There is no total agreement on the main strategies for increasing sustainability in livestock production. However, they fall into three main categories (Schader et al., 2015): (1) change the pattern of human diet; (2) feed animals less human food; and (3) improve the production efficiency. The effectiveness of the first and the second strategies has a broad consensus whereas the latter (intensification) is more questioned. All these strategies have advantages and critical points and probably, the most effective way to improve environmental sustainability, is a combination of solutions and, requires the reduction of the animal product supply, at least in developed countries (Eisler et al., 2014).

The prospect of poultry production (both conventional and alternative) is widely dependent on these options. Indeed, poultry production is well-positioned with respect to the high productive efficiency and to the change of human dietary pattern. A modern broiler is very efficient at converting feed into food (about 2:1) and the current dietary guidelines and market trend presuppose a shift from red to "white" meat (Fulginiti, 1996) also supported by the health risks retained associated with excessive consumption of red and processed meat (Bouvard et al., 2015).

However, the strategy of reducing the food-competing feed for livestock is also effective (Schader et al., 2015) and allows us to forecast a sustainable 2050 scenario for livestock production. This option hypothesizes a considerable reduction of protein from livestock and shifts the focus from livestock, as a source of high-quality nutrient, to the use of resources that cannot otherwise be used for food production. This choice is focused on reducing environmental pressures from livestock production by converging herbivore production on grassland-based systems (Eisler et al., 2014) and by reducing the amount of feedstuffs derived from cropland in both animal feeds (Foley, 2011). According to this prediction, the 2050 scenario shows a significant reduction in poultry production (−141%), whereas the reference 2050 scenario (applying the current trend of growth) would give a relevant increase (108%) for poultry (Table 14.1). In this depicted scenarios, the alternative poultry system (using pasture and high levels of alternative feedstuffs; see Section 14.3.4) could contribute, even a small part, to the global sustainability of livestock.

TABLE 14.1 Different Scenarios for Poultry Production (Billion Animals)

Current Situation: Base Year	2050: Reference Scenario	2050: Food−Not Feed
17.56	33.85	5.19

Source: Modified from Schader, C., Muller, A., El-Hage Scialabba, N., Hecht, J., Isensee, A., Erb, K.H., et al., 2015. Impacts of feeding less food-competing feedstuffs to livestock on global food system sustainability. J. R. Soc. Interface 12, 91−99.

Besides the global livestock scenario, the comparison between intensive and alternative poultry systems gives conflicting results. Bokkers and De Boer (2009), while investigating mechanisms to minimize the "carbon footprint" of farms, indicated that intensive poultry production can be more sustainable than extensive; the same is true for Van der Sluis (2007) and Mengesha (2011) which assess that intensive poultry production has less impact on global warming than organic or free-range systems. On the contrary, it was found that alternative poultry farming systems have lower environmental impacts (Castellini et al., 2006, 2012; Boggia et al., 2010), but serious concerns also exist regarding the long-term sustainability of intensive poultry systems (Acosta-Alba et al., 2012; Lindsey, 2012; Zhang et al., 2012).

Most of these discrepancies are due to data source, adoption of methods for measuring the environmental impact (e.g., life-cycle assessment, energy, ecological footprint; Bastianoni et al., 2010) and considered boundaries.

Intensive systems generally have high environmental performances when the analysis is restricted to the production phase, whereas when overall efficiency is compared, this system appears less efficient (Eisler et al., 2014). Moreover, intensive and extensive systems contain divergent effects: free-range birds are less efficient in converting feed into meat (on average 2:1 vs 3.5:1) but, at the same time, the unit of organic feed is generally less concentrated in protein and energy (NRC, 1994) and has less of an impact on the environment (no chemical weeding and mineral fertilization of crop (Castellini et al., 2006)).

Another aspect of the intensive or extensive system is the land use. Free-range birds need additional space for foraging. Grazing may improve some meat quality traits (see Section 14.7; Fanatico et al., 2006, 2007, 2008), but the energy and protein supplied to the birds are usually low (Walker and Gordon, 2003; Rivera-Ferre et al., 2007), and the land used for grazing in free-range systems is not compensated for by a reduction in land for feed production. This additional requirement of land could be reduced by applying the principle of agroforestry, i.e., rearing chickens in already productive land (Paolotti et al., 2016). Various species of domestic animals (cattle, sheep, and goats) and plants can be raised together to complement each other, creating mutually beneficial relationships (CRA website, 2014).

In this way, two or more different productions could be performed on the same area having advantages both for the birds (protection from weather, predators) and for the trees (weeding, organic fertilization, and pest control). Birds and pasture offer mutual benefits: they improve the soil quality by adding organic matter even though there is no unanimity on how much benefit they get from pasture (Berton and Mudd, 2009). Poultry can also control both insect pests and weeds while providing a new revenue stream for the farm. Poultry that are free to roam act as miniature manure spreaders that fertilize the soil. They turn and mix soil and manure as they scratch for insects and worms, increasing organic matter and improving soil fertility.

14.6 SOCIAL BENEFITS AND MAIN ECONOMICAL CONSTRAINTS

According to DEFRA (2001), pastured poultry is a sustainable livestock production system that integrates well with other farm activities and can help keep more family farmers on their land.

Under intensive farming systems, the farmers are protected from market turbulence but are only workers of big National or International suppliers with no authority on their own farm. Under alternative farming systems, the farmers generally have the direct control of the poultry farm. Pastured poultry production generally has lower entry costs and is thus more attractive for smaller farmers (Laux, 2012). Accordingly, there are social benefits of raising pastured poultry, such as family work ethics, community involvement, and improving lifestyles.

Although requiring some lower resource inputs and generally considered secondary to other agricultural activities by smallholder farmers, this type of production has an important contribution in supplying additional family income (Sossidou et al., 2010).

The production cost of pastured-poultry meat is greater than that of conventionally reared poultry, but pastured-poultry meat production is growing in some countries/areas due to good consumer demand at premium prices.

According to EU policy, it is a sustainable livestock production system that integrates well with other farm enterprises with environmental, economical, and social benefits for farmers and society.

The number of organic livestock increased during the last decade, and the market for organic meat products is expected to grow significantly (Sahota, 2009). Organic poultry is considered to be one of the first foods for consumers who are just beginning to purchase organic produce. One of the principal beliefs is that organic foods are safer than conventional foods, and consumers base this belief on the prohibition of pesticides and chemicals in the production of organic foods.

Consumers demanding pastured-poultry meat are generally willing to pay more for the system, which includes raising poultry on pasture to deliver a

product that is considered by many consumers to be healthier and tastier, as well as more environmentally sound: in one word more "natural." Consumers have different interpretations of the term "natural" but it usually includes flocks reared on pasture which eat only nonmedicated feeds. Some organic consumers believe that pastured poultry meat has better nutritive value and taste. Some look for an old-fashioned flavor, others are interested in alternative poultry for welfare reasons or because they consider the organic method to be a more environmentally sound way to produce poultry (Castellini et al., 2008; Chaveiro et al., 2008; Napolitano et al., 2013).

An example of success, deriving from the pastured-poultry system, is the Label Rouge in France. Label Rouge began 40 years ago as a movement led by some pioneer farmers, and it now accounts for about 25%–30% of poultry sales in spite of its high price—twice the price of conventional poultry. This program provides premium products to consumers, increases farmers' income, and strengthens the rural development. It consists of many producer-oriented alliances which produce and market their own products under a common label certified by a third party. It emphasizes quality attributes such as taste and food safety, and free-range production practices.

However, in some other countries/areas, the development of alternative poultry production and the demand for organic poultry meat stagnate mainly due to high price differentials with conventional meat, and limited knowledge and awareness about the organic method by consumers.

Moreover, it must be taken into account that there is an increased preference in consumers toward local and more sustainable products (Adams and Salois, 2010). Organic poultry meat does not always achieve these trends, and most of the organic poultry meat is produced (see Section 14.2) and marketed through the mainstream conventional channels. This trend of organic production into conventional marketing channels and production systems has been well-documented and is known as "conventionalization" of the organic production (Zoiopoulos and Hadjigeorgiou, 2013). The use of fast-growing birds and the "little as possible" pasture use is an example of such a strategy. As a consequence, despite being organic, this production does not always provide consumers with products that are as high-quality, local, and sustainable as they desire, nor positively impact environmental protection and rural development. Therefore, to fulfill these requirements, organic products should include the added values—already discussed—which are intrinsic in the consumer perception (Schleenbecker and Hamm, 2013; Castellini et al., 2012).

14.7 NUTRITIONAL CHARACTERISTICS OF POULTRY MEAT REARED UNDER ALTERNATIVE SYSTEMS

Although the growth of the organic food market is propelled by the perception of consumers that organic products as healthier and safer (Sundrum, 2001),

organic livestock production is not specifically designed for higher nutritional quality, and the effects of organic system on meat quality are side effects due to the pasture and the genetic strain used (see Section 14.1). Although the effects of pasture on meat quality in free-range systems has been assessed by some solid studies (Sales, 2014), many other aspects remain largely undetermined.

Meat from pasture-raised poultry may contain some nutritional benefits compared to standard broiler meat. Robertson et al. (1966) found that the meat of free-range birds contains more thiamine than the meat of birds raised indoors. Additionally, access to pastures may contribute to flavor and some forages and herbs may result in distinctive flavors (Gordon and Charles, 2002; Ponte et al., 2008).

As mentioned before, pasture is only a modest supplier of energy and protein for growing birds, but it also furnishes the birds with several bioactive compounds, such as xanthophylls, antioxidants, and hypocholesterolemic and anticarcinogenic compounds (Ponte et al., 2004).

Apart from the increase in antioxidants (carotenoids, vitamin E and C, chlorophylls, polyphenols), free-range chickens can provide vitamin D3-enriched foods from animal sources. Chicken hens have four-fold higher vitamin D3 in their eggs due to exposure to sunshine (ultraviolet (UV) rays) (Kühn et al. 2014). The same enrichment has been found in meat with UVb radiation comparable to that found in free-range systems (Schutkowski et al., 2013).

The intake of grass and microfauna, connected with a different ability of slow-growing birds to metabolize essential fatty acids, produces meat with high nutritional value (Dal Bosco et al., 2012).

In Western countries, the dietary recommendations for humans foresee a decreasing intake of saturated fatty acids replacing them with PUFAs, particularly long chain n−3 PUFAs (LC-PUFAs). A high percentage of n−3 LC-PUFAs are present in fish products, but the world fish stocks are limited and fish farming is scarcely sustainable (Bostock et al., 2010). Thus, it could be appropriate to enrich common foods (milk, cheese, meat, eggs) with n−3 LC-PUFAs because the food habits of humans are difficult to change, and people consuming low amounts of fish are expected to continue to consume low amounts of fish even in future.

The standard poultry meat has a low fat content and safe fatty-acid profile; the possibility to further increase n−3 LC-PUFA content in the meat could represent an important goal for human nutrition.

Two main possibilities exist for enhancing the n−3 LC-PUFAs in poultry meat: the addition of a precursor (α-linolenic acid; ALA) and/or the direct supplementation of LC-PUFAs (fish oil; Lopez-Ferrer, 2001a,b). The method of enriching meat is very important because the economic, ecologic, and nutritional impacts of this enrichment depend on it.

Rymer and Given (2005), reviewing the literature for improving the lipid profile of poultry meat, showed that the concentration of ALA in poultry

meat can be increased by increasing its level in the diet anyway; the supplementation with the precursor does not result in a relevant increase in n−3 LC-PUFAs (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) (depending on genetic strain). The concentration of EPA and DHA in meat is also increased when the diet is supplemented with n−3 LC-PUFAs independently of genetic strains. The strategy of adding fish oil to poultry diets has some drawbacks. The addition of fish oil to the poultry diets is not very sound because fish oil is expensive, the efficiency of the enrichment is very low (1 g fish only results in <1% LC-PUFA increase in edible tissues; Rymer and Given, 2005) and the meat could also be less stable (Jahan et al., 2004), with possible high concentrations of heavy metals.

As affirmed, the precursor strategy (supplementation of ALA) has a lower expectation of n−3 LC-PUFA increase but is much more physiological. This dietary plan appears largely affected by the n−3/n−6 ratio of the diet (Rymer and Given, 2005) and by the enzymatic pattern (genetically determined) required to elongate and desaturate ALA to LC-PUFA. The main limiting points of this metabolic pathway are Δ6/Δ5 and the specific elongases (Gregory et al., 2012). Dal Bosco et al. (2014) show that the fatty acid profile of breast meat is affected by poultry genotype (very slow growing, slow growing, and fast growing). Also the fatness and the indexes of lipid metabolism are largely related to genotype, as slow-growing strains have higher elongase, and Δ5/Δ6 desaturase indexes accompanied by a lower Δ9 desaturase. The main differences in the fatty acid profile are observed in saturated fatty acids, PUFAs, and also in the different fractions of n−3 and n−6 PUFAs. Slow-growing and very-slow-growing breeds have double conversion of ALA to its long-chain derivatives EPA and DHA (Castellini et al., 2015).

Boschetti et al. (2016) confirm a genotype-related expression of the desaturating enzymes Δ-5,-6,-9 (in liver) in very-slow-, slow-, and fast-growing chickens and in the fatty acid composition of meat. Slow- and very-slow-growing birds show a greater expression and activity of Δ-5 and -6 genes and consequently higher n−3 LC-PUFA content in the breast meat than fast-growing birds. Therefore, slow-growing strains have higher conversion rates of ALA to LC-PUFA. The exact metabolic reason for this trend is not known and it seems common even to other animal species (rabbit; Castellini et al. 2016b). One hypothesis is that, according to the theory of resources allocation, which postulates that selection for higher yield reduces the metabolisms and behaviors are more expensive in terms of energy (feeding and kinetic behavior, reproduction activity) and not directly involved in the selected traits. In summary, the efficacy of the ALA strategy is adequate in slow-growing (largely used in alternative systems) but not in fast-growing strains.

Concerning the sensorial properties of the meat, Ponte et al. (2008) observed that members of a consumer panel preferred the meat from grazing broilers having access to pasture of clover (*Trifolium subterraneum* or *repens*)

compared to the control conventional group. Napolitano et al. (2013), comparing conventional with organic chickens from slow-growing or fast-growing strains, showed that trained panelists discriminated breast meat from different sources. Meat from conventional birds is perceived as more tender and fibrous than organic meat, and leaves more residues, whereas slow-growing broilers are less juicy and fibrous than fast-growing ones. However, untrained consumers are not able to discriminate between chicken breasts from different sources. Indeed, a consumer panel from southern, central, and northern Italy did not find differences for perceived liking (tasting without information) whereas expected liking (information without tasting) was higher for organic and actual liking (tasting with information) of organic breasts moved toward the expectancy. Consumer liking seems more affected by the information given on the organic production system than by the sensory differences.

Castellini et al. (2002a) assessed the effects of organic production on broiler carcass and meat quality. Organic poultry systems produce carcasses with higher breast and drumstick percentages and lower levels of abdominal fat. The muscles have lower ultimate pH and water-holding capacity. Instead, cooking loss, lightness values, shear values, iron content, n−3 PUFA, and extent of oxidative processes are higher. As already mentioned, a negative aspect of the organic system is the higher level of lipid peroxidation in the muscles, probably due to greater physical activity.

Horsted et al. (2012) compared the sensory profiles of breast meat from five broiler products: two conventional products and three organic slow-growing genotypes (I657, L40, and K8) reared in an apple orchard, and found relevant differences between conventional and organic broilers. The study indicates that aroma and taste attributes are more important for the assessors than meat "tenderness" for the overall liking of broiler meat.

Meat quality is also affected by preslaughter management practices. The increased production and utilization of epinephrine and glucocorticoids in animals exposed to antemortem stressors can affect postmortem metabolism and meat quality (Kannan et al., 1997).

Castellini et al. (2016a) evaluated the effects of transport length and chicken genotypes reared under free-range conditions, and observed that the transport length (0 vs 4 h) affects the fatty acid profile of breast muscle, with a decrease of PUFAs, antioxidants content (α-tocotrienol, α,δ−tocopherol, and lutein + zeaxanthin of breast meat), and an increase in the TBARS value. Such trends are more relevant in more active birds.

Accordingly, the alternative rearing system requires not only adapted poultry genotypes, and a proper management of pasture, but also a particular care in the peri-mortem phase due to the more active behavior of slow-growing strains which, during this lag-phase, could have a greater consumption of glycogen and an unsatisfactory meat acidification (Berri et al., 2005). There continues to be a lack of viable poultry processing for small poultry flocks as evidenced by

Angioloni et al. (2015) in the USA and also in Europe (Ranucci et al., 2015). This situation supports the need for more affordable poultry processing options to serve small-scale farmers because the lack of abattoirs strongly discourages the producers. There are few examples of Mobile Poultry Processing Units (MPPUs) which can be shared by different farmers to reduce costs; they go directly to the farms avoiding the stress of long transport times in animals.

MPPUs are available in the USA (O'Bryan et al., 2014) and recently also in Europe (MPPUs are in use for "spent" hens in Sweden (Carlsson et al., 2007) and in Italy, Umbria region; Figs. 14.3, 14.4). MPPUs generally operate under exemptions that exclude them from continuous inspections by the Sanitary Authorities, but they are still required to meet all sanitation and wholesomeness requirements. The exemption implies a limitation on the number of animals slaughtered per year (around 10,000 in Italy) and in the local boundaries where the poultry is sold (in Italy it is the province of the farms and adjacent areas).

In the USA there is also the possibility to have an MPPU controlled by the US Department of Agriculture; either way, regulated or unregulated, the MPPU may be an affordable and efficient solution to add value to small flock production.

Small-scale poultry farmers, both organic and free-range, may benefit from the opportunities associated with mobile processing.



FIGURE 14.3 Picture and layout of mobile poultry processing unit.

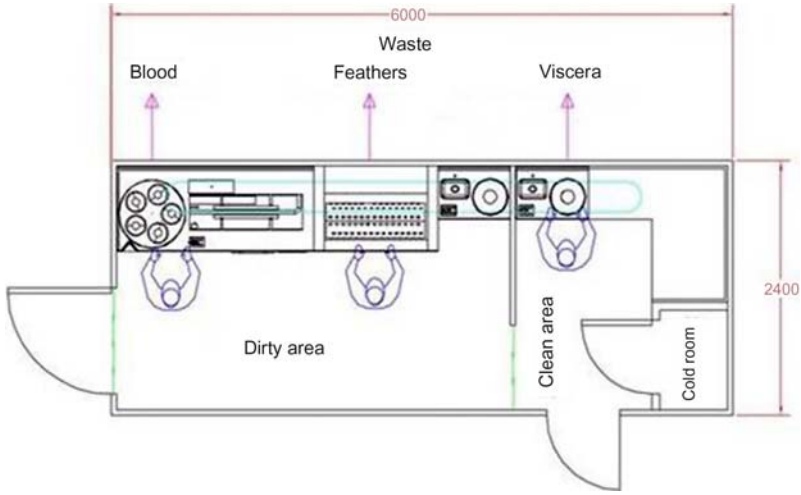


FIGURE 14.4 Layout of mobile poultry processing unit.

14.8 CONCLUSION

In summary, pasture availability and genetic strain are key issues to maintain high health and welfare standards in the poultry meat alternative production system. An optimal combination of genetic and pasture management can ensure the best results in terms of environmental sustainability without compromising animal welfare and costs, thanks to the improvement of the nutritional value and the sale price of the meat. On the contrary, when inappropriate genetic/environment interactions are employed, some serious welfare problems such as greater rates of body lesions in organic and free-range systems still have to be expected. The chicken wellbeing is likely to increase if more birds are encouraged to go outside. Consequently, the qualitative traits of meat are positively affected by pasture availability mainly in term of nutritive value.

If poultry producers, companies and policy makers do not take into account these aspects, the growth of the alternative poultry sector, as well as their positive externalities, will be limited (Escribano et al., 2015).

Once these results are achieved, to maintain a high quality level, painstakingly achieved through an accurate management of the animals in the phases of capture, transport, and slaughter; errors in these phases, as well as in the handling of carcasses and in meat processing can affect all the work done in the rearing phases, favoring the lipid oxidative processes and the loss of nutritional characteristics.

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Covering ethics, genetics, animal welfare, and health impact, *Poultry Quality Evaluation* focuses on what constitutes poultry quality in the minds of consumers, marketers, and producers in the 21st century.

In the 21st century, the general understanding of what constitutes poultry quality in the minds of consumers, marketers, and producers has changed. Traditional measures such as texture, water-holding, color, flavor and aroma, safety and microbiology, and processing characteristics are still important. However, additional quality attributes now significantly affect the purchasing intentions of consumers in many countries. These qualities include animal welfare, the impacts of poultry meat on human health, organic or free-range status and ethical poultry production, among others.

Poultry Quality Evaluation provides a new reference source that covers these market aspects with the same scientific authority as texts on traditional poultry meat quality values.

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About the Editors

Dr. Massimiliano Petracci has been an associate professor at the University of Bologna since 2014. His research interest is poultry and rabbit production with particular emphasis on the nutritional value and technological properties of poultry meat as affected by genotype, housing, feeding, and preslaughter factors. He is the author of more than 60 peer-reviewed articles and an invited speaker at several international congresses. He chairs the Working Group 5 "Poultry Meat Quality" of the European Federation of the World's Poultry Science Association.

Dr. Cécile Berri is current Director of the INRA Avian Research Unit in Nouzilly, France. Her main scientific contributions are the unraveling of the molecular pathways involved in the control of muscle glycogen and postmortem metabolism and the study of the impact of genetics and animal nutrition on poultry meat quality. She is the author of more than 60 peer-reviewed articles, an invited speaker at many international congresses, and the coinventor of a genetic marker that controls chicken breast meat color.

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