VOLUME 2

ADVANCES IN FOOD SCIENCE AND NUTRITION

EDITED BY
Visakh P.M., Laura B. Iturriaga,
Pablo Ribotta, and Sabu Thomas

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100 Cummings Center, Suite 541J
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Publishers at Scrivener
Martin Scrivener (martin@scrivenerpublishing.com)
Phillip Carmical (pcarmical@scrivenerpublishing.com)

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Preface

Advances in Food Science and Nutrition summarizes many of the recent technical research accomplishments in the areas of potato production, composition and starch processing; milk and different types of milk products; processing and preservation of meat, poultry and seafood; food ingredients; fruits and fruit processing; antioxidant activity of phytochemicals and their method of analysis; indispensable tools in food science and nutrition; transformations of food flavor due to elaboration of industrial processing; new trends in sensory characterization of food products, and; ultrasound applications in food technology. As the title indicates, the book emphasizes various aspects of the advances in food science and nutrition and their different applications for the food sciences and scientific community. It is written in a systematic and comprehensive manner and all recent advances are discussed in detail. It is very important to mention that till now, there have not been many books published on this topic.

In this sense, the content of this book is unique. It presents up-to-date records on major findings and observations in the field, and is intended to serve as a "one stop" reference resource for related important research accomplishments. The various chapters of the book are contributed by prominent researchers from industry, academia and government/private research laboratories around the world. Therefore, it will be a very valuable reference source for university and college faculties, professionals, post-doctoral research fellows, senior graduate students, food science technologists and researchers from R&D laboratories working in the area of food science and nutrition.

The first chapter on food chemistry and technology is an overview of the contents of the book. This chapter is essential for beginners since it provides a thorough understanding of the basics of food science.

Chapter 2 discusses potatoes and their production, composition and starch processing. The chemical composition of potatoes is explained along with the effects that cultivar, location, growth, fertilizer applications, maturity at harvest, and storage conditions have on them. A survey on milk and different types of milk products, their processing and preservation are covered in Chapter 3. Among the other topics discussed by the authors are milk production and quality.

Chapter 4 discusses processing and preservation of meat, poultry and seafood. Numerous topics are explored by the authors such as food quality characteristics; deterioration and microbial contamination; physical and chemical methods of preservation; preliminary processes; control of moisture and temperature; radiation and other technologies; various methods and compounds; microbiological contributions to meat; hurdle combinations of methods, and; atmosphere inside packaging.

Useful terminology and definitions are found in Chapter 5 on food ingredients. Also covered are food additives, novel and natural plant-based ingredients, and properties and applications of plant-derived ingredients. Chapter 6 discusses fruits and fruit processing. Included in the many subtopics are the effects of low temperature on fruits; modified and controlled atmosphere storage; modified atmosphere packaging; edible coatings; factors affecting fruit conservation methods; traditional preservation methods, and; modern preservation methods with minimal processing.

The authors of Chapter 7 on antioxidant activity of phytochemicals and their method of analysis address the importance of antioxidants in human health. Also addressed are natural antioxidants; methods used to measure total antioxidant activity; problems in comparing various methods of antioxidant activity and discrepancies over their measurement, and; methods for antioxidant phytochemical analysis.

Chapter 8 on indispensable tools in food science and nutrition is a thorough discussion enhanced by many reviews in recent research works. Topics are presented on food safety from farm to plate; foodborne pathogens; probiotics in food; the pros and cons of genetically modified (GM) foods; bioavailability of nutrients, and; food safety regulations.

The important topic of transformations of food flavor due to elaboration of industrial processing is covered in Chapter 9. Topics discussed are aroma compounds; chemical reactions that contribute

food flavor; the Maillard reaction; formation of flavor compounds in the Maillard reaction and kinetics and factors influencing it; flavor from lipids; flavors formed via fermentation, and; special processes used in the industrial production of flavor. Chapter 10 discusses new trends in sensory characterization of food products. Explained in the various topics are descriptive analysis; methodologies based on specific attributes; methodologies that provide a verbal description of the products; methods based on the comparison with references, and; comparison of the methodologies.

The effect of food processing on bioactive compounds is presented in Chapter 11. The author includes many of the recent advances related to the topics of bioactive compounds; reactive oxygen species; antioxidant defenses against reactive oxygen (RO); bioactive compounds and natural antioxidants; processing of foods containing bioactive components; effect of postharvest handling methods and shelf life determination; methods for the determination of antioxidants; methods for measuring the oxidation of an oil or food sample; techniques involving bioactive compound determination, and; high performance liquid chromatography (HPLC).

Advancements in storage technologies for fresh fruits are presented in Chapter 12. Different techniques for food storage are discussed such as methylcyclopropene (1-MCP) based storage technology; palladium-based ethylene adsorbers; ultra low oxygen (ULO) storage technology; dynamic controlled atmosphere (DCA) storage technology; microcontrolled atmosphere (MCA) and bulk modified atmosphere packaging (MAP) technologies; nitric oxide based technology, and; biosensors.

The final chapter is on ultrasound applications in food technology. The equipment used in the applications, combined processes and effects on safety and quality parameters are discussed. Some of the specific topics are ultrasound application in equipment design for improving processing efficiency; food preservation applications; enzymes and microorganisms, and; ultrasound effects on food quality attributes.

Finally, the editors would like to express their sincere gratitude to all the contributors of this book, who were an excellent support throughout the successful completion of this venture. We are grateful to them for the commitment and the sincerity they have shown towards their contribution to the book. Without their enthusiasm and support, the compilation of a book series could not have been possible. We would like to thank all the reviewers who have taken

their valuable time to make critical comments on each chapter. We also thank the publisher Wiley-Scrivener for recognizing the demand for such a book, for realizing the increasing importance of the area of food science and nutrition, and for starting a new project in which not many other publishers are yet involved.

Visakh. P. M
Laura B.Iturriaga
Pablo Daniel Ribotta

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Recent Advances in Food Science and Nutrition: State of Art, New Challenges and Opportunities

Visakh. P.M.[1,2,*], Laura B. Iturriaga[3] and Pablo Daniel Ribotta[4]

[1]*Centre for Nanoscience and Nanotechnology, Mahatma Gandhi University, Kottayam, Kerala, India*
[2]*School of Chemical Sciences, Mahatma Gandhi University, Kottayam, Kerala, India*
[3]*Institute of Chemical Sciences, Faculty of Agronomy , National University of Santiago del Estero, Santiago del Estero, Argentina*
[4]*Department of Science and Technology, National University of Córdoba, Córdoba , Argentina*

Abstract

This chapter presents a brief account on various topics concerning food science and nutrition. Also presented are different parameters within food science and nutrition such as potato production, composition and starch processing; milk and different types of milk products; processing and preservation of meat, poultry and seafood; food ingredients; fruits and fruit processing; antioxidant acivity of phytochemicals and their method of analysis; indispensable tools in food science and nutrition; transformations of food flavour due to elaborative industrial processing; trends in sensory characterization of food products; effects of food processing on bioactive compounds; recent advances in storage technologies for fresh fruits and; ultrasound applications in food technology, etc. Also discussed are recent technical research accomplishments in the area that have immense structural possibilities for chemical and mechanical

**Corresponding author*: visagam143@gmail.com

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modifications to generate novel properties, functions and applications, especially in food science and nutrition.

Keywords: Food science, nutrition, potato production, milk products, food ingredients, fruit processing, food flavour, bioactive compounds

1.1 Potato Production, Composition and Starch Processing

The chemical composition of potatoes varies with cultivar, location, growth, fertilizer applications, maturity at harvest, and storage conditions. Potato tubers contain about 80% water and 20% dry matter. Starch constitutes the major portion of the dry matter. Total starch content of different potato varieties can vary greatly from about 9 to 23% of the fresh weight [1]. These values represent 66–80% of potato dry matter as starch [2]. Fresh potatoes contain 10–18% starch, 1–7% total sugars, 1–2% protein, 0.5% fibre, 0.1–0.5% lipids, 30 mg/100g vitamin C and 1–3 mg/100g glycoalkaloids [3]. Large-sized russet potatoes provide higher calories, protein, carbohydrates, sugars and fibre and lipids as compared to their counterpart small-sized and medium-sized potatoes. Large-sized russet, red and white potatoes have protein content of 7.9, 6.97 and 6.2 g/potato, respectively, while small-sized russet, red and white potatoes had protein content of 3.6, 3.2 and 2.9 g/potato, respectively. The accumulation of starch in potatoes is dependent on genotype, environmental conditions and genotype-environment interaction [4]. The temperature during tuber growth also influences starch characteristics [5]. The starch accumulation showed a positive relation with tuber growth and the optimum temperatures for tuber bulking and starch content in tubers are between temperatures of 15 and 21°C [6]. Higher yields of potatoes were obtained under short days and cool night temperatures as compared to a long days and warm night environment [7]. Ingram and McCloud [8] found temperatures of 14–16°C to be optimal for tuber formation. The composition of potatoes also varied with the application of fertilizers [9]. Inorganic nitrogen (N) as ammonium nitrate is the most often used fertilizer applied to potatoes for promoting vegetative growth, delaying tuber initiation and increasing tuber size and yield. The rate of N recommended dose varies with the variety, soil type and nature of previous crops grown. The sugar content in tubers

increased in response to N deprivation by up to 100% compared to those produced with adequate application of fertilizer [10]. The adequately fertilized plants with N usually produced potatoes that had lower reducing sugar concentration at harvest [11]. Increased N fertilizer has also been shown to cause a rise in free amino acid concentrations [12], while S deficiency has been found to cause an increase in the concentrations of sugars [13].

Potatoes are a poor source of proteins and lipids. They contribute only a small portion of total daily protein intake, as they contain relatively small amounts of protein (~2g/100g in fresh potatoes). The primary storage proteins in potato tubers are patatins, which account for 40% of the soluble protein content [14]. The molecular mass of patatin monomer ranges between 39 and 43 kDa [15, 16]. Patatin is interesting for use in food and biotechnological applications as it has good functional, nutritional and biochemical properties [17]. Asparagine is the most abundant free amino acid in potato tubers, typically accounting for approximately one-third of the total free amino acid pool [18, 19]. Potato lipid content varies between 0.1–0.5% (fresh weight basis). Boiled potato cooked in skin contains about 0.1 g total lipids, 0.026 g total saturated fatty acids, 0.002 g total monounsaturated fatty acids, and 0.043 g total polyunsaturated fatty acids per 100 g [20]. Polyunsaturated fatty acids account for a higher proportion than monosaturated and saturated fatty acids in potato lipids. The predominant fatty acid of potato tuber was linoleic acid accounting for ~50% of total fatty acids, followed by linolenic acid and palmitic acid, each contributing to approximately 20% [21]. Phospholipids and glycoglycerolipids were the predominant fraction of lipids in potato tubers [22]. Phosphatidylcholine was reported to be a major phospholipid (30.7 mol% of the total polar or complex lipids), followed by phosphatidylethanolamine (19.6%), phosphatidylinositol (9.3%), phosphatidic acid (3.2%), phosphatidylserine (1.5%), phosphatidylglycerol (1.2%), and diphosphatidylglycerol (cardiolipin) (0.7%) [23].

Starch that escapes hydrolysis by the amylolytic enzymes in the small intestine and passes to the large bowel is defined as resistant starch [24]. Phosphorus content in starch was positively correlated to resistant starch (RS) content in native starch and to the slowly digestible starch content in the starch gel. The RS content is related to the rate of starch digestion by amylolytic enzymes [25]. The RS content is influenced by numerous factors, including the source of starch and its composition, phosphorus content [26], ratio

of amylose and amylopectin [27], chain length distribution of amylopectin [28], and processing and storage conditions.

1.2 Milk and Different Types of Milk Products

Milk is a white liquid produced by the mammary glands of mammals for feeding their young. It is secreted as a natural process in the mammary glands after parturition of the newborn. According to the Food and Agriculture Organization (FAO) and World Health Organization (WHO) Codex Alimentarius Commission, milk is a substrate, whether processed, semi-processed or raw, that is intended for human consumption. Humans have a long tradition of consuming milk produced by animals, and cow's milk is the most popular milk to be consumed in both developed and developing countries. Goat's milk is also consumed in some regions with a high preference in some parts of Europe, particularly in France and Italy, since breeding of dairy sheep and goats is common there. There are significant roles of goat milk and its products in human nutrition including [29] feeding more starving and malnourished people in the developing world; [30] treating people afflicted with cow milk allergies and gastro-intestinal disorders, which is a significant segment in many populations of developed countries [31], and; filling the gastronomic needs of certain consumers, which is a growing market share in many developed countries.

Milk is a complete food for the young animals and is consumed by humans due to its high nutritional value with all the nutrients that are good for human health. Milk, excluding water, contains complete nutrients that are a source of protein, lipids, carbohydrates, vitamins and minerals. It also contains several bioactive compounds such as immunoglobulins, hormones, cytokines and nucleotides. On the other side, milk has been reported to contain the most common food allergens including β-lactoglobulin, α-lactalbumin and caseins. Several technologies of milk processing such as heat treatment, enzymatic hydrolysis and fermentation by lactic acid bacteria (LAB) is one strategy to destroy or eliminate the allergens of milk. Research aimed at producing hypoallergenic milk is of interest for future development. Milk is a highly nutritious food that provides complete nutritional needs for humans of all ages. The consumption of milk either as milk per se or milk products varies considerably among regions depending on tradition, availability, price and other reasons.

Organic milk production is based on organic principles and objectives including naturalness and the recycling of nutrients [32]. Consumer interest in organic milk has been growing recently. The boost in organic milk sales is part of a wider growing interest in organic products, which resulted in an average annual growth rate of retail sales of organic food of nearly 18 percent between 1998 and 2005 [33]. However, the consumption of organic milk is still controversial. People may turn to organic milk for health benefit purposes, or environmental and animal rights' issues. So far, when evaluating the health claims research does not support a health advantage of organic over conventional milk for any segment of the population [34]. Milk and milk products represent an important food for human as they provide valuable nutrients for all ages. Research on the development of new milk products has been widely carried out with the application of new technologies, and these products can be categorized as functional foods.

1.3 Processing and Preservation of Meat, Poultry and Seafood

Meat is defined as the flesh of animals consumed as food, which is mostly the muscle tissue of an animal. For centuries, meat, poultry, seafood and their derived products have constituted some of the most important foods consumed worldwide. The human body has complex nutritional requirements that must be fulfilled, and those food products are one of the major important sources of a wide variety of essential nutrients in the human diet. Animal muscle is typically composed of 60–80% water, 18–20% protein, 0.5–19% lipids, 1–1.5% minerals and a trace of carbohydrate [35–37]. However, this composition varies extremely, mainly in the lipid content (0.5–19%), which in turn affects the amount of water present in the tissues. Animal characteristics (e.g., species, breed, age, gender and weight), nutritional regime (type of feed and feeding), environmental conditions and geographical factors, hygienic practices and disease control programs, may affect meat characteristics.

High protein content is one of the most important characteristics of meat. It plays an important role in the human diet as a source of essential amino acids such as leucine, lysine, threonine, methionine and tryptophan, which are required for cellular maintenance, growth, and functioning of the human body [38, 39].

Nowadays, fish is more recognized as a supplier of micronutrients, minerals and essential fatty acids, than by its protein value. Vitamins A and D, calcium, phosphorus, magnesium, iron, zinc, selenium, fluorine and iodine are some examples of the essential micronutrients and minerals for the human diet that are present in fish [40].

Once the muscles of animals are nutrient-enriched matrixes, they provide a suitable environment for proliferation of spoilage microorganisms, becoming one of muscle foods major sources of pathogens that may cause foodborne diseases in humans. Food safety is a priority for authorities and consumers worldwide. Therefore adequate preservation processes must be applied in order to assure the safety and quality of food. The application of methods and technologies to foods that alter their raw state and characteristics is designated by food processing. Food processing has three major goals: to make food safe while providing products with the highest quality attributes, to make food into forms that are more convenient or more appellative to be consumed, and to extend shelf life [41]. Temperature plays an important role in food processing: high temperatures are crucial for microbial death or inactivation (safety point of view), whereas low temperatures are often applied for long-term food preservation, preventing microbial growth and retarding reactions of quality alterations, from a joint perspective of safety and quality.

Food processing dates back to ancient times. Foods were sun dried, fermented, salted, smoked and frozen in glacier waters aimed at longer preservation. Alterations in food taste, texture and appearance caused by processing were later found to be also appealing. Food processing technologies were greatly developed after World War II, with the expansion of a consumer society in developed countries. Processes such as spray drying, freeze drying and irradiation were innovations of that time, as well as the introduction of sweeteners, food colouring agents and preservatives such as sodium benzoate. Over the past years, there has been a growing interest in the alteration and control of the atmosphere within food packages aimed at food preservation and shelf-life extension. The development of technologies and related equipment were fundamental for the advances of food processing operations [42], namely cook-chill, vacuum packaging systems, ionizing irradiation, phage technology, high pressure, and hydrodynamic shockwave.

1.4 Food Ingredients

Ingredients and additives, such as those called 'functional food ingredients' and 'specialty ingredients', are continuously being developed to meet the requirements of consumers and/or food manufacturers. However, attention should be paid to the limitations/drawbacks and regulatory issues of these new ingredients. A new dietary ingredient is generally deemed adulterated under Section 402(f) of the United States Federal Food, Drug, and Cosmetic Act (FD&C Act), unless it meets one of the following requirements: 1) The dietary supplement contains only dietary ingredients which have been present in the food supply as an article used for food in a form in which the food has not been chemically altered; 2) There is a history of use or other evidence of safety establishing that the dietary ingredient when used under the conditions recommended or suggested in the labelling of the dietary supplement will reasonably be expected to be safe and at least 75 days before being introduced or delivered for introduction into interstate commerce, the manufacturer or distributor of the dietary ingredient or dietary supplement provides the Secretary with information, including any citation to published articles, which is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such dietary ingredient will reasonably be expected to be safe.

Food additives are substances added to food, other than a basic foodstuff, to preserve a food, enhance stability of a food, and/or facilitate food processing. Sometimes, even if manufacturing conditions are satisfactory, there is a necessity to use chemical additives to impart desired physical properties to the end product. There are six main categories of food additives: colourants, flavouring agents, nutritional additives, preservatives, texturing agents and other miscellaneous additives. An international numbering system (INS) has been developed for food additives by the Codex Alimentarius Commission Committee on Food Additives and Contaminants, and the E system by the European Union.

1.5 Fruits and Fruit Processing

Fruits and vegetables include a miscellaneous group of plant foods that vary significantly in content of energy and nutrients. Fruits are essential components of a balanced human diet, representing a good

source of macro- and micronutrients such as sugars, vitamins, minerals, organic acids, water soluble pigments, dietary fibre, and phytochemicals [43]. Low intake of fruits and vegetables is among the top 10 risk factors contributing to mortality, according to evidence presented in the World Health Report 2003. Fruits and vegetables, as part of the daily diet, could help to prevent major non-communicable diseases. Moreover, eating a variety of vegetables and fruits clearly ensures an adequate intake of most micronutrients, dietary fibres and a host of essential non-nutrient substances. The dietary fibre, and fibre intake associated with fruit consumption is linked to lower incidence of cardiovascular disease and obesity. Fruits also provide the diet with important phytochemicals such as polyphenols, which are secondary plant metabolites with potential beneficial health effects such as antioxidant activity and antimicrobial, antiviral and anti-inflammatory properties. A WHO/FAO expert consultation report on diet, nutrition and prevention of chronic diseases sets population nutrient goals and recommends the minimum intake of 400 g of fruits and vegetables per day for the prevention of chronic pathologies such as heart disease, cancer, diabetes and obesity.

Fruits, together with vegetables, are fundamental sources of water-soluble vitamins (vitamin C and group B vitamins), provitamin A, phytosterols, dietary fibres, and minerals for the human diet. Scientific evidences encouraged the consumption of fruits and vegetables to prevent chronic pathologies such as hypertension [44], coronary heart diseases and the risk of stroke [45]. Recently, the population of developed countries has modified its nutritional habits as a consequence of new life styles. In fact, many studies have reported that the new eating habits related to this life style are causing health problems. An example is the relationship established between fast food with obesity and type-2 diabetes [46]. Unfortunately, the daily intake of vegetables and fruits is estimated to be lower than the doses (400 g, excluding potatoes and other starchy tubers) recommended by the World Health Organization (WHO), and Food and Agriculture Organization (FAO). The food industry is concerned with the elaboration of healthier food products without forgetting the importance of taste and flavour, since they are very important characteristics to consumers [47].

The total production of fruits in the world in 2010 was around 609,213,509 metric tons. An approximate distribution according to the earth's surface is Oceans 0.5–1%; Europe 8–12%; Africa

11–13%; America 22–35% and Asia 41–51% [48]. In accordance with this distribution of production, and due to the season-dependent production of the majority of fruits, it is important to apply fruit conservation strategies to guarantee consumption of these fruits worldwide.

There are many nutritional similarities between fruits and vegetables, but there is one important difference with respect to conservation, since most fruits are more acidic than the majority of vegetables. This difference significantly affects the way that these two types of crops are processed because food pathogenic bacteria cannot grow in acidic fruit products. The majority of fruits are consumed fresh or are minimally industrially processed, and include canned, dried, juice, paste, salad, sauce and soup preparations. Minimally processed and, especially, fresh fruits have a short shelf life since they are subjected to rapid microbial spoilage, and, in some cases, to contamination by pathogens. Cooking and pasteurization, as well as the addition of chemical preservatives, are the main technological options that guarantee safe vegetables and fruits, but these options also bring about a number of not always desirable changes in their physical characteristics and chemical composition [49–51]. To reduce these drawbacks, some novel technologies like high-hydrostatic pressure processing, irradiation and pulsed-electric fields [52], as well as new packaging systems and the use of natural antimicrobial preservatives [53], have been reported as alternatives in recent years. The latest techniques have lower detrimental effects on fruits than the conventional strategies (heat, freezing, etc.), and have attained considerable interest from the related fruit industries.

1.6 Antioxidant Activity of Phytochemicals and Their Method of Analysis

The concept of antioxidant activity of unprocessed and processed foods is gaining significant momentum and emerging as an important parameter to assess the quality of the product worldwide. With the expansion of the world global market and fierce competition amongst various multinational companies, the parameter of antioxidant activity will soon secure its place in nutritional labelling with accompanying regulatory guidelines. In this context development of a practical method of determining the antioxidant activity

for industrial use will become imperative. This will give a further boost to the exploitation of fruits and vegetables and development of nutraceuticals and beverages. In this respect, it is of paramount importance to develop analytical methods to quantify antioxidants in foods of plant origin.

Antioxidants can inhibit or retard oxidation in two ways: either by scavenging free radicals, where the compound is described as a *primary antioxidant*, or by a mechanism that does not involve direct free radical scavenging, where the compound is a *secondary antioxidant*. Primary antioxidants include phenolic compounds such as vitamin E (α-tocopherol). These components are consumed during the induction period. Secondary antioxidants function by various mechanisms including binding of metal ions, oxygen scavenging, hydroperoxide conversion to non-radical species, absorbing UV radiation or deactivating singlet oxygen. Normally, secondary antioxidants exhibit antioxidant activity only when a second minor component is present. For example, sequestering agents such as citric acid are effective only in the presence of metal ions, and reducing agents such as ascorbic acid are effective in the presence of tocopherols or other primary antioxidants

1.7 Indispensable Tools in Food Science and Nutrition

The modern consumer demands a wide variety of foods from different parts of the world. This has significantly impacted the import and export of a variety of food products. Moreover, dinners that would usually take hours to prepare, can now be prepared in a few minutes. Economically, this increasing demand has created new opportunities. At the same time, this also exposes these food products to transportation contamination, adulteration and spoilage. Moreover, climate change has become an increasing factor that is impacting foodborne pathogens. Ensuring that these food products retain their nutritional content and flavour through this process has become an increasingly important factor. Food science is an interdisciplinary science that uses chemical engineering, molecular biology, microbiology, environmental science, botany, statistics and informatics to study all the steps involved from food production to consumption, which include harvesting, processing, flavouring,

packaging, and storing conditions up to the consumption of food; essentially from farm to the dinner table.

Food science is constantly evolving with new advances in food safety, and the production of genetically modified (GM) plants. Ultimately, this is a global responsibility and everyone from the producers to the consumers plays an active role. The challenges of readily available food products, climate change, foodborne pathogens and bioavailability of nutrients, when coupled with the development of sophisticated genetic recombination techniques and novel testing methods to detect foodborne pathogens, provide us with the tools we need to ensure a safe and nutritious food supply.

1.8 Transformation of Food Flavours Due to Industrial Processing Elaboration

Flavours represent an important challenge in terms of process engineering because they cover a very broad range of sensory and thermophysical characteristics. Besides, they are sometimes unstable and are perceived by human beings on the basis of very complex, extremely nonlinear mechanisms [54, 55]. Commercial flavourings are complex mixtures of solvents, pure flavouring agents and natural isolates, which in turn consist of flavouring agents. The aroma threshold value is the lowest concentration of a certain odor compound that is perceivable by the human sense of smell. The threshold of a chemical compound is determined in part by its shape, polarity, partial charges and molecular mass.

Finally, a problem that makes flavour difficult to study is its instability during analysis and sample preparation. Foods are a dynamic system that can change even while stored awaiting analysis. Consequently, the all analytical process must be very controlled to obtain representative and reproducible results. If possible, the flavour isolation process must be strong enough to extract the analytes and, at the same time be sufficiently careful to not modify these. Unfortunately, once we have considered each of the points above and attain some instrumental result of the flavour compounds in a certain food, we are left with the enormous question of attempting to determine the importance of each compound to the perceived flavour. During the past 50 years this topic has been the subject of immeasurable research articles.

Humans are capable of recognizing five main taste qualities: sour, sweet, bitter, salty, and savory (umami), and maybe several sub-qualities. This number of taste qualities is comparatively small if is compared with the number of chemical compounds that elicit taste. In the last years, with the advent of sophistical instruments to separate and measure aroma compounds, the researchers have an increasing knowledge about the flavour and aroma of compounds, and thier interaction and behavior in different foods. Even so, there is much to learn about flavour. The new technological processes used in food elaboration are other important topics in food flavouring, making it a dynamic subject matter. The multitude of interactions between all components and environmental factors (such as temperature, water content, etc.) give a final sensorial quality to foods and beverages.

1.9 New Trends in Sensory Characterization of Food Products

One of the most common applications of sensory characterization is during new product development and product reformulation. At these stages it is usual for product formulation and processing conditions to be systematically varied following an experimental design in order to generate a series of prototype products [56]. In this context, sensory characterization enables the product developer to evaluate how formulation and processing variables affect the sensory characteristics of the prototypes to determine how close the prototypes are from the target product to be developed, and to take decisions based on objective sensory information.

Furthermore, during the implementation of sensory quality assurance programs, sensory characterization plays a key role in defining specifications or quality standards for the sensory characteristics of food products, as well as for establishing specifications for physicochemical properties that are related to specific sensory characteristics [57].

There is increasing interest in gathering sensory information directly from the target consumers of food products instead of by the more technical descriptions provided by trained assessors [58]. The most common approach to product optimization is to ask consumers to rate their liking of a large set of products and characterize the sensory properties of those products using a trained assessors'

panel. Then, both data sets are combined using regression analysis to identify the sensory characteristics of the consumers' ideal product [59]. In these approaches consumers are only asked about their liking, and therefore information about how they perceive the sensory characteristics of the products is not gathered. However, trained assessors could describe the product differently or take into account attributes that may be irrelevant for consumers [60]. Considering that the best way to understand consumer preferences might be consumer data [61], getting consumer feedback about the sensory characteristics of food products has become of great interest in the last decade.

1.10 Effect of Food Processing on Bioactive Compounds

Researchers have investigated antioxidants and found that various kinds of antioxidants can protect humans from oxidative stress [62–65]. They have suggested that though synthetic antioxidants have been developed and used in practice, natural antioxidants can be more potent, efficient and safe.

Fruits and vegetables are a good source of natural antioxidants. For example, vitamin E in fruit and nuts is the major lipid-soluble antioxidant present in low density lipoprotein (LDL) and can prevent the formation of lipid peroxides. Vitamin C in fruits and vegetables can also scavenge free radicals in cytoplasm [66]. However, although β-carotene, a vitamin A precursor, is contained in LDL, the antioxidant mechanism is not yet known.

The other bioactive compounds found in fruits and vegetables, such as flavonoids, are also beneficial. Aged garlic extract which contains high flavonoids, such as s-allylcysterine and N-alpha-(1-deoxy)-D-fructos-1-yl)-L-arginine, was studied in men and shown to improve endothelial function, decrease LDL oxidation and inflammatory factors, and slow the development of experimental atherosclerosis. A number of research studies have been done that have focused on the effect of storage conditions on the quality of food. Dhemre and Waskar [67] suggested that storage of mangoes in a cooling chamber could maintain the quality and market acceptability. Boukobza and Taylor [68] worked on the effect of pre- and postharvest treatments on the level of volatile compounds in fresh tomato quality and found that varietal and seasonal factors have a significant effect on the loss

of volatile compounds, whereas the study of chilling storage caused a reduction in the levels of volatile components, as did short-term, high-temperature storage (45°C for 15 hours).

McGlynn *et al.* [69] studied sanitizing dip as a postharvest treatment on the quality of fresh-cut watermelon. They found that a pre-cut sanitizing dip reduced about one to two log cycles in initial aerobic and coliform bacterial counts. This is expected to extend the shelf life of fresh cut melon when stored at 4°C for up to 14 days.

However, there is limited information on the effect of postharvest treatments on the bioactive components of fruit and vegetable, especially in native plants [70]. Sommano *et al.* [71] indicated to maintain a good level of bioactive compounds from native food ingredients throughout food processing, freeze drying is recommended.

1.11 Recent Advances in Storage Technologies for Fresh Fruits

Fruits are an essential constituent of the human diet as they are rich sources of vitamins, minerals, bioactive compounds, and dietary fibre. Scientific evidence is increasing in favour of roles of phytochemicals in preventing and controlling several chronic diseases and lifestyle disorders.

Most fruits are perennial and produced seasonally. The narrow harvest window for many fruits is the cause of wastage and market gluts, lowers returns to fruit growers, shortens the period of fruit availability to the consumers and puts seasonal pressure on processing industries. The storage of fruits is therefore indispensable when addressing these issues. Globalisation has given tremendous impetus to the international fruit trade, facilitating commercial movement of fruits from one part of the world to the other. This ensures availability of all types of fruits to the consumer throughout the year. The counter-season production in the northern and southern hemispheres is another advantage in the fresh fruit trade. Transport is a major activity in the fruit supply chain where storage technologies can be employed to preserve fruit quality to meet expectations of shippers, retailers, and consumers. The typical shipping time for fruits depends on the mode of transport and it varies from hours for the air transport to weeks for the marine shipment.

The commercial application of biosensors has had a significant impact in a number of areas, particularly in the field of medical

diagnostics. Disposable blood glucose biosensors, frequently used by diabetes sufferers to monitor their blood sugar levels, make up the vast majority of the current total biosensor market. Undoubtedly, this trend will continue, yet opportunities to exploit biosensor technology in areas other than medical diagnostics do exist. One such industry where biosensor technology will be further exploited is in the food industry. Currently, however, food testing represents a very small percentage of the total market, but with advances in sensor longevity and stability and with new applications on the horizon, biosensors for food diagnostics are set to expand. Traditionally, the food industry has taken a very conservative approach to the introduction of biosensors but would benefit from improvements in quality control, safety, and traceability that these relatively inexpensive devices can offer.

Recent advances in storage technologies have impacted the postharvest industry worldwide. The introduction of 1-methylcyclopropene (1-MCP)-based technology has provided several benefits to the apple industry. The scope of application of 1-MCP in other fruits is increasing as the registration of this compound for edible horticultural commodities has already been made in many countries and is imminent in several others. The combination of 1-MCP with controlled/modified atmospheres with static O_2 and DCA is a promising hybrid technology that can improve the storage stability of fruits. **Ultra-low oxygen** (ULO) and dynamic controlled atmosphere (DCA) systems offer additional advantages in terms of maintaining fruit quality. The application of ULO, DCA, and 1-MCP has been mainly focused on apple fruit. However, there is a huge scope for extending their applications in other fruits. It is also probable that ethylene inhibiting/suppressing technologies other than cyclopropenes will also contribute to extending storage and shelf life. The bulk modified atmosphere packaging (MAP) and pallet covers are well integrated into the supply chain of soft fruits such as strawberries. These technologies are simple, cost-effective and pose minimal operational difficulties. The new fumigants such as nitric oxide are still at an experimental stage and may find application in the near future. The choice and adoption of a storage technology or diagnostic device (e.g. biosensor) for a particular fruit is strongly influenced by the return on investment factor in addition to sustainability issues. Researchers and the industry have successfully confronted the challenges of the past and can capitalise on the opportunities that lie ahead, so that the fresh fruit industry

will continue to contribute to the economic and social wellbeing of growers and consumers for many decades to come.

1.12 Ultrasound Applications in Food Technology

Ultrasound applications alone or in combination with other treatments have received great attention due to their potential to produce safe food products with higher nutritional and sensory quality. Work has been conducted in different food products and treatment combinations to confirm the clear benefits of the ultrasonic technologies and their main drawbacks. In terms of consumer acceptability, the application of emerging technologies such as ultrasound have raised much lower levels of concern than 'irradiation', 'genetic engineering', and other more controversial technologies [72].

Ultrasound is a form of energy generated by sound waves equal to or above 20000 vibrations per second (20000 Hz or 20 kHz), generally above frequencies that can be detected by the human ear, that is able to travel through gas, liquid and solid materials [73, 74].

Ultrasounds are produced by an electric apparatus, the generator, which can be controlled to create vibrations at a desired frequency. The ultrasound power supply converts the 50/60 Hz line voltage into high frequency electrical energy which is then transmitted to the piezoelectric transducer within the converter, and transformed into mechanical vibrations. These vibrations are intensified by a probe, which when inserted in a liquid medium create pressure waves [75–79]. These transducers may be strategically placed on the sidewall or at the bottom of a tank or can be directly immersed in the liquid [80].

The ultrasound treatments can be combined with and/or replace the traditional thermal processes with an objective of producing safer food products with higher nutritional and sensory quality. Besides allowing a successful reduction of treatment temperature and showing a high versatility that is adaptable to different processing operations, a lack of standardization in ultrasound equipment configuration, operating frequencies and power levels makes meaningful comparisons between different studies difficult to achieve. Further investigations in different food products and treatment combinations with duly controlled process conditions are required in order to obtain a better understanding of the benefits

and drawbacks of ultrasound treatments. Such information will allow economically feasible production-scale implementation of ultrasound technologies in the food industry.

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2

Potato: Production, Composition and Starch Processing

Narpinder Singh[1,*], Amritpal Kaur[1], Khetan Shevkani[1] and Rajarathnam Ezekiel[2]

[1]*Department of Food Science and Technology, Guru Nanak Dev University, Punjab, India*
[2]*Central Potato Research Institute, Shimla, India*

Abstract

Potato is the fourth most important crop with global production of around 373 million tonnes. Potato is a cheap source of calories, vitamin C and minerals like calcium (Cu), magnesium (Mg), phosphorus (P), iron (Fe), iodine (I), etc. The chemical composition of potatoes varies with genotype, colour, environmental conditions, fertilizer applications, soil type, postharvest storage conditions and processing conditions. Potatoes also contain several phytochemicals (phenolics, flavonoids and carotenoids) which have beneficial effects on human health. Tubers with dark-coloured flesh (dark yellow, red and purple) have higher contents of the beneficial phytochemicals. Fried potato products, such as French fries and chips contain high levels of acrylamide. Acrylamide formation depends upon the amount of reducing sugars and free asparagines and the processing conditions. Potato products are considered high glycemic foods and their consumption over longer periods may increase the risk of obesity and related chronic diseases. Starch is the main carbohydrate of potatoes and constitutes up to 80% of dry matter. Potato starch has an edge over other starches (corn, wheat and rice) because of low gelatinization temperature, high paste viscosity and formation of translucent paste. The uniqueness of potato starch is attributed to its large granular size and the presence of phosphate groups linked to amylopectin.

Keywords: Potato, composition, minerals, vitamins, acrylamide, starch

**Corresponding author*: narpinders@yahoo.com

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2.1 Introduction

Potato (*Solanum tuberosum*) is the fourth most important vegetable crop of the world after rice, wheat and maize. Potato tubers are specialized stems of potato plant that grow under the soil surface. Potatoes can be cultivated in various climates and soil types, and are a relatively inexpensive source of calories. Potatoes are mainly processed into frozen French fries, chips, snacks and dehydrated products [1]. Potato production by major potato producing countries between 1985 and 2011 is presented in Table 2.1. Global potato production is around 373 million tonnes from an area of about 19 million hectares with the global average yield of around 17809.1 kg/Ha [2]. China, India, the Russian Federation, Ukraine and the USA are the major potato producing countries. China is the largest producer and consumer of potatoes in the world followed by India and the Russian Federation. China, India and the Russian Federation produced 88.35, 42.33 and 32.68 million tones of potato during the year 2011 [2]. China and India potato production was 32.03 and 14.77 million tonnes, respectively, in 1990, and that increased to 70.9 and 28.78 million tonnes, respectively, in 2005. The export of potatoes between 2000 and 2010 by different countries is shown in Table 2.2. France, the Netherlands, Germany, Belgium and Canada are top potato exporters. France, the Netherlands and Germany exported 2.31, 1.88 and 1.59 million tones of potatoes during 2010. France exported 1.1 and 1.48 million tonnes of potatoes in 2000 and 2005, respectively, while the Netherland exported 13.4 and 15.0 million tonnes in those years. Germany exported 13.5, 12.8 and 15.9 million tonnes, respectively, of potatoes during 2000, 2005 and 2010. The export of potatoes by most potato exporting countries has increased in the last decade.

2.2 Composition

Chemical composition of potato varies with cultivar, location, growth, fertilizer applications, maturity at harvest, and storage conditions. Potato tubers contain about 80% water and 20% dry matter. Starch constitutes the major portion of the dry matter. Total starch content of different potato varieties can vary greatly from about 9 to 23% of the fresh weight [3]. These values represent 66–80% of potato dry matter as starch [4]. Fresh potatoes contain 1–18% starch, 1–7% total sugars, 1–2% protein, 0.5% fibre, 0.1–0.5%

Table 2.1 Production of potatoes between 1985 and 2011 (data in tones).

Country	2011	2010	2005	2000	1995	1990	1985
China	88350220	74799084	70906729	66318167	45983531	32031189	26792976
India	42339400	36577300	28787700	25000100	17401300	14770800	12570600
Russian Federation	32681500	21140500	37279800	33979500	39909100	NA	NA
Ukraine	24248000	18705000	28787700	19838100	14729400	NA	NA
USA	19361500	18016200	19222700	23294000	20122000	18239000	18443000
Germany	11800000	10201900	11624200	13694300	10888100	14471000	21053800
Poland	8196700	8765960	10369300	24232400	24891300	36312800	36546100
Bangladesh	8326390	7930000	4855380	2933000	NA	NA	NA
Belarus	7721040	7831110	8184950	8717800	9504000	NA	NA
Netherlands	7333470	6843530	6777000	8227000	7340000	7036000	7149610
France	8016230	6582190	6604600	6434050	5839000	4754420	7787000
United Kingdom	6115000	6045000	5979000	6636000	6404000	6467000	6892000

Source: FAOSTAT [2]

Table 2.2 Export of potatoes by different countries during 2000–2010 (data in tones).

Country	2010	2005	2000
France	2318680	1488230	1109320
Netherlands	1883601	1504176	1347739
Germany	1592520	1281175	1354049
Belgium	784858	931319	876725
Canada	493657	433509	449563
USA	386176	289728	324479
UK	336699	219062	186505
Egypt	298557	392178	156630
Iran	262973	174747	NA
China	258683	244690	NA
Pakistan	245329	NA	86423

Source: FAOSTAT [2]

lipids, 30 mg/100g vitamin C and 1–3 mg/100g glycoalkaloids [5]. The composition of large, medium and small russet, red and white potatoes is shown in Table 2.3. Large-sized russet potatoes provide higher calories, protein, carbohydrates, sugars and fibre and lipids as compared to their counterpart small-sized and medium-sized potatoes. Large-sized russet, red and white potatoes have protein content of 7.9, 6.97 and 6.2 g/potato, respectively, while small-sized russet, red and white potatoes had protein content of 3.6, 3.2 and 2.9 g/potato, respectively. The russet potatoes have higher protein, carbohydrates and lower fibre and total lipid content compared to red and white potatoes (Table 2.3). The accumulation of starch in potatoes is dependent on genotype, environmental conditions and genotype-environment interaction [6]. The temperature during tuber growth also influences starch characteristics [7]. The starch accumulation showed a positive relation with tuber growth and the optimum temperatures for tuber bulking and starch content in tubers were between temperatures of 15 and 21°C [8]. Higher yields of potatoes were obtained under short days and cool night temperatures as compared to long days and warm night's environment [9].

Table 2.3 Proximate composition of russet, red and white potatoes.

Potato	Water (g)	Energy (kcal)	Protein (g)	Total lipid (g)	Carbohydrates, by difference (g)	Fibre, total (g)	Sugars (g)
Large russet	289.96	292	7.90	0.30	66.68	4.8	2.29
Large red	298.74	258	6.97	0.52	58.67	6.3	4.76
Large white	301.03	2555	6.20	0.37	57.97	8.9	4.24
Medium russet	167.38	168	4.56	0.17	38.49	2.8	1.32
Medium red	172.44	149	4.03	0.30	33.87	3.6	2.75
Medium white	173.77	147	3.58	0.21	33.46	5.1	2.45
Small russet	133.59	134	3.64	0.17	30.72	2.2	1.05
Small red	137.63	119	3.21	0.24	27.03	2.9	2.19
Small white	138.69	117	2.86	0.17	26.71	4.1	1.95

Large potato 369 g; medium potato 213 g; small red and russet potato 170g; small white potato 92g Source USDA [22].

Ingram and McCloud [10] found temperatures of 14–16°C to be optimal for tuber formation. The composition of potatoes also varied with the application of fertilizers [11]. Inorganic nitrogen (N) as ammonium nitrate is the most often used fertilizer applied to potatoes for promoting vegetative growth, delaying tuber initiation and for increasing tuber size and yield [11]. The rate of N recommended dose varies with variety, soil type and nature of previous crops grown. The sugar content in tubers increased in response to N deprivation by up to 100% compared to those produced with adequate application of fertilizer [12]. The adequately fertilized plants with N usually produced potatoes that had lower reducing sugar concentration at harvest [13]. Increased N fertilizer has also been shown to cause a rise in free amino acid concentrations [14], while S deficiency has been found to cause an increase in the concentrations of sugars [15]. S deficiency also affected free amino acid concentrations and has been reported to be variety-dependent, because in some varieties accumulation of free glutamine in response to S deficiency was reported, while in one variety accumulation of both free asparagine and free glutamine was observed [11].

Potatoes are a poor source of proteins and lipids. Potatoes only contribute to a small portion of total daily protein intake as they contain a relatively small amount of proteins (~2g/100g in fresh potatoes). The primary storage proteins in potato tubers are patatins, which account for 40% of the soluble protein content [16]. The molecular mass of patatin monomer ranges between 39 and 43 kDa [17, 18]. Patatin is of interest for use in food and biotechnological applications as it has good functional, nutritional and biochemical properties [19]. Asparagine is the most abundant free amino acid in potato tubers, typically accounting for approximately one-third of the total free amino acid pool [14, 20, 21]. Potato lipid content varies between 0.1–0.5% (fresh weight basis). Boiled potato cooked in skin contains about 0.1 g total lipids, 0.026 g total saturated fatty acids, 0.002 g total monounsaturated fatty acids, and 0.043 g total polyunsaturated fatty acids per 100 g [22]. Polyunsaturated fatty acids account for a higher proportion than monosaturated and saturated fatty acids in potato lipids (Table 2.4). The predominant fatty acid of potato tuber was linoleic acid accounting for ~50% of total fatty acids, followed by linolenic acid and palmitic acid, each contributing to approximately 20% [23]. Phospholipids and glycoglycerolipids, were the predominant fraction of lipids in potato tubers [24]. Phosphatidylcholine were reported to be major phospholipid (30.7 mol% of the total polar or complex lipids), followed by phosphatidylethanolamine (19.6%),

Table 2.4 Fatty acid composition of russet, red and white potatoes.

Potato	Total saturated (g)	Total monounsaturated (g)	Total polyunsaturated (g)
Large russet	0.096	0.007	0.159
Large red	0.129	0.011	0.218
Large white	0.096	0.007	0.159
Medium russet	0.055	0.004	0.092
Medium red	0.075	0.006	0.126
Medium white	0.055	0.004	0.092
Small russet	0.044	0.003	0.073
Small red	0.060	0.005	0.100
Small white	0.044	0.003	0.073

Large potato 369 g; medium potato 213 g; small red and russet potato 170g; small white potato 92g. Source USDA [22].

phosphatidylinositol (9.3%), phosphatidic acid (3.2%), phosphatidylserine (1.5%), phosphatidylglycerol (1.2%), and diphosphatidylglycerol (cardiolipin) (0.7%) [25].

Potatoes are considered a good source of the major minerals calcium (Ca), magnesium (Mg), phosphorus (P), and potassium (K), and the trace minerals iron (Fe) and Zinc (Zn) [26]. Potato has been described as a 'major supplier' of Cu, Mg, P, Fe, I, and Zn but not Ca based on the recommended daily intake [27]. Mineral content of potatoes is dependent upon many factors such as genotype, colour, soil type, cultural practices, etc. Large-sized russet, red and white potatoes have relatively higher Ca, I, Mg, P, K, Na and Zn content as compared to their counterpart small-sized russet, red and white potatoes (Table 2.5). P, Mg and Ca content of large-sized russet, red and white potatoes ranged from 203 to 229, 77 to 85 and 33 to 48 mg, respectively. On the other hand, small-sized russet, red and white potatoes showed P, Mg and Ca content from 94 to 105, 36 to 39 and 15 to 22 mg/potato, respectively. The K content of large-sized russet, red and white potatoes ranged between 1502 and 1679 mg/potato. The Zn content of large-sized russet, red and white potatoes was higher than similar potato types having a small size. In addition to

Table 2.5 Mineral content of russet, red and white potatoes.

Potato	Calcium (mg)	Iron (mg)	Magnesium (mg)	Phosphorus (mg)	Potassium (mg)	Sodium (mg)	Zinc (mg)
Large russet	48	3.17	85	203	1539	18	1.07
Large red	37	2.69	81	225	1679	66	1.22
Large white	33	1.92	77	229	1502	59	1.07
Medium russet	28	1.83	49	117	888	11	0.62
Medium red	21	1.5	47	130	969	38	0.70
Medium white	19	1.11	45	132	867	34	0.62
Small russet	22	1.46	39	94	709	8	0.49
Small red	17	1.24	37	104	774	31	0.56
Small white	15	0.88	36	105	692	27	0.49

Large potato 369 g; medium potato 213 g; small red and russet potato 170g; small white potato 92g. Source USDA [22].

substantial mineral content, potatoes contain a relatively low concentration of anti-nutrients that decrease mineral bioavailability, such as oxalates and phytates [28]. Potatoes have a high concentration of compounds such as ascorbate, β-carotene, organic acids, and cysteine-rich polypeptides that promote bioavailability of minerals [29]. Potatoes are an important source of different dietary mineral elements. They provide 18% of the recommended dietary allowance (RDA) of potassium, 6% of iron, phosphorus and magnesium, and 2% of calcium and zinc [30]. The mineral elements are concentrated in the skin portion of potatoes [31]. Thus, retention of mineral elements is high in boiled potatoes cooked with skin [32].

The vitamin composition of different potato types are shown in Table 2.6. Potatoes are a well-known source of vitamin C that may provide about 36% of the RDA [30]. White potatoes had higher vitamin C compared to russet and red potatoes (Table 2.6). Large-sized russet, red and white potatoes have higher thiamine, riboflavin, niacin, vitamin B6, folate, vitamin A and K as compared to their counterpart medium- and small-sized russet, red and white potatoes. The cold storage of potatoes rapidly decreases vitamin C content and loss could be up to 60%, while wounding of potatoes can increase the vitamin level up to 400% [33, 34]. The cooking of potatoes with skin using microwaving, steaming, baking and boiling have shown a negligible loss of vitamin C [30].

Potato contains several phytochemicals such as phenolics, flavonoids, polyamines, and carotenoids, which are highly desirable in the diet because of their beneficial effects on human health. The concentration and stability of these constituents were affected by several factors such as genotype, agronomic factors, postharvest storage, cooking and processing conditions [35]. Potatoes were considered the third most important source of phenols after apples and oranges [36]. The main phenols of potato are neochloroenic acid (0.1–43.7 mg/100 g dry matter), chlorogenic acid (22–473 mg/100 g dry matter), caffeic acid (0.5–47.6 mg/100 g dry matter) and rutinose (0.29–6.91 mg/100 g dry matter) [37]. Carotenoid content of fresh potatoes vary from 50 to 350 µg/100 g in white-fleshed cultivars and between 800–2000 µg/100 g in dark-yellow-fleshed cultivars (Brown 2008). The flavonoid content of fresh potatoes ranged from 200 to 300 µg/g, wherein the purple- and red-fleshed potatoes had twice the flavonoid concentration of white-fleshed cultivars [38]. The total anthocyanin content of red- and purple-fleshed fresh potatoes was 22 mg/100 g and 368 mg/100 g, respectively [38].

Table 2.6 Vitamin content of russet, red and white potatoes.

Potato	Vitamin C (mg)	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Folate (µg DFE)	Vitamin A (IU)	Vitamin K (µg)
Large russet	21.0	0.303	0.122	3.819	1.273	52	4	6.6
Large red	31.7	0.229	0.114	4.240	0.627	66	26	10.7
Large white	72.7	0.262	0.125	3.934	0.749	66	30	5.9
Medium russet	12.1	0.175	0.070	2.205	0.735	30	2	3.8
Medium red	18.3	0.173	0.066	2.447	0.362	38	15	6.2
Medium white	42.0	0.151	0.072	2.271	0.432	38	17	3.4
Small russet	9.7	0.139	0.056	1.759	0.586	24	2	3.1
Small red	14.6	0.138	0.053	1.953	0.289	31	12	4.9
Small white	33.5	0.121	0.058	1.812	0.345	31	14	2.7

Large potato 369 g; medium potato 213 g; small red and russet potato 170g; small white potato 92g. Source USDA [22].

Potatoes constitute a substantial portion of total starch intake and exert variable glycemic response. Potatoes have been classified as a high glycemic index (GI) food that cause high postprandial glycemia, and consumption over the long term may increase the risk of obesity and chronic diseases such as type-2 diabetes and cardiovascular disease [39]. GI is a relative measure of the postprandial glycemia evoked by foods that are significant sources of carbohydrate [40]. Foods having a GI value above 70 are classified as high GI, while those with a GI between 56 and 69 as medium GI, and foods that have a GI of ≤ 55 are classified as low GI [41]. GI values of potatoes vary over a large range depending on the cooking method, processing and meal composition [42–44]. GI values of potatoes and potato-based products vary depending on the varietal differences, storage conditions and post-cooking handling [39]. Soh and Brand-Miller [45] studied the effect of cooking methods on the GI of potatoes and reported that only little differences were observed in GI when potatoes were boiled and mashed versus boiled and cut into large pieces. Leeman *et al.* [46] investigated the effects of cold storage and vinegar addition on glycaemic responses of potato meals in healthy subjects. The cold storage of boiled potatoes was reported to increase resistant starch (portion of starch that is not digested in human body) content from 3.3 to 5.2% (starch basis), while the GI of cold potatoes added with vinegar was significantly reduced by 43% compared with the GI of freshly boiled potatoes. The lower GI of cooked and cooled potatoes have been attributed to the retrogradation of gelatinized starch during refrigerated storage, while the presence of acetic acid in the meal delayed the gastric emptying rate and thus reduced postprandial glycaemia [47, 48].

Acrylamide formation in carbohydrate rich food during processing has attracted considerable attention worldwide [49]. The acrylamide is a neurotoxin and genotoxin [50]. Fried potato products are major contributors of dietary acrylamide. According to the European Food Safety Authority the indicative levels of acrylamide in chips and French fries is 1000 and 600 ppb, respectively. The acrylamide formation in food occurs through Maillard reaction, a complex series of non-enzymatic reactions between amino acids and reducing sugars. This reaction occurs during the processing of potatoes at high temperatures. Frying, baking, and roasting are the examples of such processes. The Maillard reaction produces a large number of compounds, many of which impart colour, aroma, and flavour [51–53]. The acrylamide is formed only when the

asparagine is available for participation in reaction at final stages [54, 55]. The free asparagine and reducing sugars are regarded as the most important precursors for the formation of acrylamide. Potatoes have considerable amounts of these precursors and, therefore, fried potato products such as French fries and chips, have been reported to contain high levels of acrylamide [56, 57]. Sucrose, a non-reducing sugar, does not directly contribute to acrylamide formation, while it may undergo hydrolysis to form glucose and fructose during high temperature frying, thus, the reducing sugars produced take part in the reaction to form acrylamide [58]. Acrylamide formation depends upon the amount of reducing sugars and free asparagines and the cooking conditions such as temperature, time, pH and the surface-to-volume ratio of the food materials [59, 60]. Decreasing the pH has been suggested as a way to reduce the amount of acrylamide in processed food [61]. Pretreatment with citric acid and some organic acids decreased the pH of processed foods and was reported to be effective in reducing acrylamide levels [61]. Kita *et al.* [62] studied the effect of blanching or soaking in different acid solutions on the acrylamide content in potato crisps. The authors reported that the largest decrease of acrylamide content (90%) in crisps was obtained when potato slices were soaked in acetic acid solution for 60 min at 20°C. Soaking or blanching of potato slices in acidic solutions decreases the pH of potato juice and increases the extraction of acrylamide precursors: amino acid and reducing sugars.

2.3 Starch Production

Corn, wheat, cassava and potato are the primary sources of starch. Potato starch is preferred in many food applications over other starches because of its low gelatinization temperature, high paste viscosity and formation of translucent paste. European countries account for 80% of global potato starch production. The first process for potato starch production was reported in 1758. It involved a wet procedure and was simple, useful and generally applicable in small units [63]. The process consisted of rasping/grating, sedimentation, decanting of diluted fruit water, scraping off sludge and fibres, re-suspending of the sediment and a second sedimentation stage. After decantation and final removal of coarse particles, the

sediment was dewatered over filter cloth and dried. The modern processes involve many unit operations such as cleaning and washing of potatoes, rasping/grating, centrifugation, screening, starch refining, starch de-watering and drying [63]. An alternative potato starch technology is available that does not start with early separation of concentrated fruit water immediately after rasping. In this case, gratings pass counter-current extraction and go to de-watering of rasps. Counter-current extraction uses a two-stage system consisting of decanters, separators or hydrocyclones. The quantity of the resulting fruit water is lowered by approximately 4% because of wash water recirculation [64]. The commercial method for production of potato starch is illustrated in Figure 2.1. The potatoes are washed to remove the dust and rasped in rotary grater to disrupt the cell walls. This leads to the release of the starch. During rasping tyrosine present in the potato could be oxidized by polyphenol oxidase, which leads to the darkening of the pulp. To avoid this darkening, potato juice is quickly separated in the shortest possible time. The enzymatic darkening of potato juice is also prevented with sulfurous acid. After separation of potato juice, the pulp is washed, and centrifuged to recover a high yield of starch. The starch slurry containing fibre particles (potato tissue fragments) and the remaining components of the potato juice are separated by wet screening. The process of centrifugation and screening is repeated several times depending upon the purity of starch required. The purified starch is dewatered and finally dried.

Compared to the wet milling processes of corn and wheat, potato starch production is simple. The starch separation from potato tubers is easier since potato tissue can be ground with ease and requires less mechanical force. The economical success of a potato starch production process depends on how completely the potatoes are grated to get maximum recovery of intracellular starch. However, the disposal of process water from potato starch processing is problematic [65]. The process water contains a substantial amount of protein and nitrogenous compounds. Potato protein (patatin) can be recovered from process water. Patatin is highly soluble in water and has many food and biotechnological applications such as in the production of stable foams and emulsions [66, 67], production of food gels [68], use as antioxidative additives [4], or as an agent with biocide effects reported for its antifungal activity against plant pathogen *Phytophthora* infections [69].

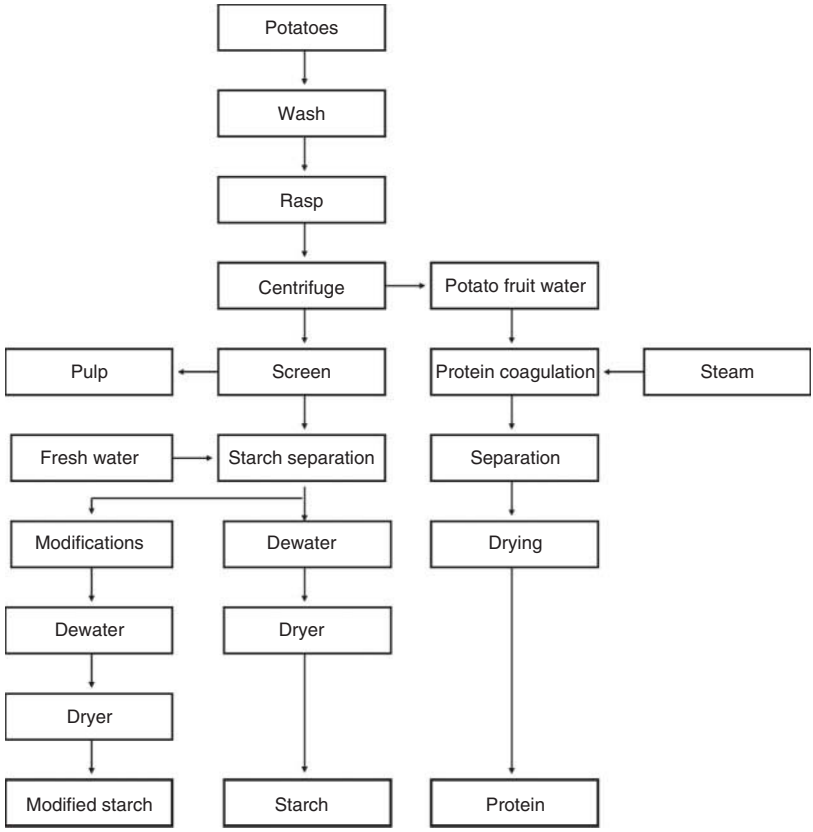

Figure 2.1 Flow diagram of potato starch processing. From Ruffer *et al*. [97]

2.4 Starch Properties

Potato starch granules are oval and irregular or cuboidal in shape with a size of approximately 10–100 μm in diameter [70]. The starch granules are composed of amylose and amylopectin. Amylose content of potato starch varies from 23% to 31%. The amylose content of the starch granules varies with the varieties and is affected by the climatic conditions and soil type during growth [70]. Waxy potatoes essentially containing mainly amylopectin and negligible amylose have also been reported [71]. Amylopectin is branched polymer of glucose with an average degree of polymerization (DP) of 21–28 [72]. On the other hand, amylose is essentially a linear polymer consisting of chains of DP in the order of 2000–5000 residues [73]. Potato starch also contains a small amount of non-carbohydrate

materials such as proteins and minerals. Unlike cereal starches, lipids are almost absent in potato starch. Potato starch also contains phosphorus in the form of phosphate linked to the amylopectin component, which is responsible for unique properties of tuber starch. The phosphorus content can range between 36 and 116 mg/100 g of potato starch with a median of 60–80 mg/100 g [74–76]. The high viscosity, transparency, water binding capacity and freeze thaw stability of potato starch have been attributed to phosphate groups esterified to the amylopectin fraction of the starch [77, 78]. Phosphorus is present as phospholipids in cereal starches which produce opaque and lower-viscosity pastes upon heating. On the other hand, upon heating potato starches produce translucent pastes. Potato starch like other starches is semi-crystalline in nature with varying levels of crystallinity. The crystallinity is exclusively associated with the amylopectin component, while the amorphous regions mainly represent amylose [79]. The packing of amylose and amylopectin within the granules has been reported to vary among the starches from different species. X-ray diffractometry is used to reveal the presence and characteristics of the crystalline structure of the starch granules [73]. The potato starches exhibit the typical B-type X-ray pattern, whereas, the cereal and legume starches showed A and C patterns, respectively [70]. When starch granules are heated in excess water, intra- and interchain hydrogen bonds between amylose and amylopectin are broken and water molecules bond to exposed hydroxyl groups. This process is termed as gelatinization. During heating the crystalline structure is disrupted and results in an increase in solubility of glucan chains (mostly amylose). Starch gelatinization is the disruption of molecular order within the starch granule. It results in granular swelling, crystallite melting, loss of birefringence, viscosity development and solubilization. The extent of swelling and solubility reflects the strength of interactions between starch chains. As the temperature increases, starch undergoes an irreversible phase transition where the native crystallinity, structural organization and birefringence are lost [80]. Differential scanning calorimeter (DSC) is widely used to evaluate gelatinization behaviour of starches. DSC permits the evaluation of starch transitions over a wide range of moisture content as well as transition temperatures and enthalpy changes during transitions. The differences in transition temperatures between the different starches are attributed to the differences in the degree of crystallinity. High transition temperatures result from a high degree of

crystallinity, which provides structural stability and makes the granule more resistant towards gelatinization [81]. Waxy potato starches exhibit higher gelatinization temperatures as well as higher enthalpies than normal potato starches due to higher crystallinity. The amylose in the normal potato starches decreases the relative amount of crystalline material in the granule and, hence, allows gelatinization at a lower temperature [82]. Lower temperature during tubers growth results in starch with higher granule size and lower gelatinization temperatures [7].

During processing, starch dispersions are subjected to high temperature and shearing conditions that affect their rheological properties as well as the final characteristics of the products. Starch gelatinization, especially granular swelling, changes the rheological properties of starch. The subsequent cooling of cooked starch pastes leads to retrogradation that further modifies the rheological properties. Rheological properties of potato starch are investigated using a Brabender visco amylograph (BVA), Rapid Visco Analyzer (RVA) and dynamic rheometer (DR). The RVA and BVA provide information on changes in viscosity of starch during heating and cooling at high shear. Pasting properties of potato starch are compared with corn, rice and kidney bean starches in Table 2.7. Potato starch showed high peak viscosity, trough viscosity, breakdown viscosity and lower pasting temperature as compared to other starches. Pasting curves of starches from different sources are illustrated in Figure 2.2. The rheological properties depend on the swelling capacity; potato starch is capable of swelling to a higher degree and has greater susceptibility to breakdown during heating. Potato starches show higher breakdown viscosity than cereal and pulse starches. Differences in the breakdown values of starches are attributed to the difference in granule rigidity, amylose content and lipid content. G' (elastic response), G'' (viscous response) and tan (G''/G') are the parameters obtained by DR. G' is a measure of the energy stored in the material, while G'' is a measure of the energy dissipated or lost per cycle of sinusoidal deformation [83]. The ratio of the energy lost to the energy stored for each cycle can be defined by tan δ, which is used to indicate the degree of elasticity. The rheological parameters of potato starch vary with the size of granules. The large and cuboidal or irregular-shaped granules in potato starch exhibit higher storage and loss modulus and lower tan δ, than the small and oval granules [84]. Nutting [85] reported that phosphate groups got ionized when starch pastes were heated and resulted in slight Coulombic

Table 2.7 Pasting properties of different starches measured using RVA.

Source	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown viscosity (cP)	Final viscosity (cP)	Setback viscosity (cP)	Pasting temperature (°C)
Potato	7247e	5072e	2175e	5671d	599b	65.5a
Corn	2205a	1182a	1023b	2458b	1276c	76.65d
Waxy corn	3028b	1901b	1127c	2109a	208a	68.1b
Rice	4145d	2796d	1349d	6542e	3746e	73.15c
Kidney bean	3118c	2488c	630a	3871c	1383d	76.35d

Values with similar alphabet did not differ significantly ($p>0.05$).

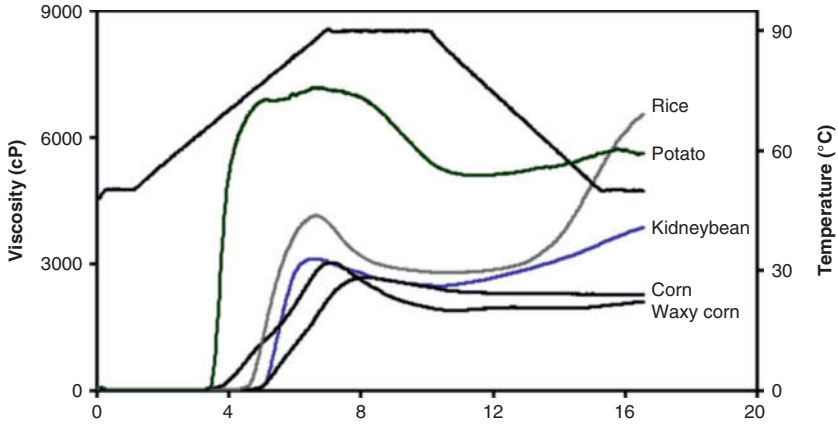

Figure 2.2 Pasting profiles of different starches measured using RVA.

repulsion causing opening of branched amylopectin molecules. This phenomenon is responsible for higher swelling, paste viscosity and breakdown viscosity of starches [86]. Therefore, potato starch granules rigidity and resistance to rupture during heating and shearing depend upon phosphate content. The starches with high phosphorous content are more easily ruptured and are less rigid than the starches with low phosphorous content. The integrity of swollen starch granules is also a major factor that determines the rheological properties of starch pastes [87]. Singh and Kaur [88] reported that large-sized potato starch granule fractions (40–80 µm) had higher amylose content and lower swelling than small-sized granule fractions (5–20 µm). G' and G'' among potato starch fractions increased in order of small, medium and large granules when subjected to temperature sweep. The breakdown in G' during heating and retrogradation during storage was also observed to be the highest for large-sized fractions and the lowest for small-sized fractions [88]. The presence of high phosphate monoester content and the absence of lipids and phospholipids in the potato starch are considered to be responsible for higher G' and G''. Cereal starches have a lower G' and G'' than potato starch. More rigid granules and the presence of phospholipids in cereal starches are responsible for their lower moduli. Amylose content significantly affects the rheological properties of the starch. Kaur *et al.* [89] reported higher moduli for potato starches having higher amylose contents. Similarly, it has also been reported that the starches isolated from waxy potatoes show lower G', G'' and higher tan δ [89]. The variation in amylopectin structure

amongst starches from different potato varieties has also been reported to affect the rheological properties of starch. Potato starches with higher amounts of short-chain amylopectin fraction (degree of polymerization, DP 6–12) were observed to show a greater change in moduli and more viscous character than other starches with lower amounts of these fractions during rheological measurements [90]. In potato starches, both the short- and long-side-chain amylopectins probably contribute to the increase in moduli; however, more so in the contribution of short-chain amylopectin [90]. During cooling of cooked starch pastes, reassociation of starch molecules takes place in a process known as retrogradation. Lu *et al.* [91] reported that potato starch with a high amylose level and higher phosphorus content showed enhanced retrogradation, associated with a well-formed and rigid gel structure and more ordered structure, compared to starch with lower phosphorus content.

Starch that escapes hydrolysis by the amylolytic enzymes in the small intestine and passes to the large bowel is defined as resistant starch [92]. Phosphorus content in starch was positively correlated to resistant starch (RS) content in native starch and to the slowly digestible starch content in the starch gel. RS content is related to the rate of starch digestion by amylolytic enzymes [93]. RS content is influenced by numerous factors, including the source of starch and its composition, phosphorus content [94], ratio of amylose and amylopectin [95], chain length distribution of amylopectin [96], and processing and storage conditions [93].

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3

Milk and Different Types of Milk Products

Yantyati Widyastuti[1,*] and Andi Febrisiantosa[2]

[1]Research Center for Biotechnology, Indonesian Institute of Sciences, Cibinong, Indonesia
[2]Technical Implementation Unit for Development of Chemical Engineering Processes, Indonesian Institute of Sciences, Yogyakarta, Indonesia

Abstract
Humans have a long tradition of consuming milk produced by animals. Milk is used as raw materials for several milk products such as liquid milk as beverage, skim milk, cream, butter and ice cream. Fermentation of milk using lactic acid bacteria produces yoghurt and cheese, two of the most popular products. Fermented milk are reported as milk products having health properties.

Keywords: Milk, milk product, fermentation, lactic acid bacteria, functional food

3.1 Introduction

Milk is a white liquid produced by the mammary glands of mammals for feeding their young [1]. It is secreted as a natural process in the mammary glands after parturition of the newborn. According to the FAO/WHO Codex Alimentarius Commission, milk is substrate whether processed, semi-processed or raw, that is intended for human consumption. Humans have a long tradition of consuming milk produced by animals, and cow's milk is the most popular milk consumed in both developed and developing countries.

**Corresponding author*: yantyati.widyastuti@lipi.go.id

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Goat's milk is also consumed in some regions and is highly preferable in some parts of Europe, particularly in France and Italy, since the breeding of dairy sheep and goats is common there. The significant role of goat's milk and its products in human nutrition include: 1) feeding more starving and malnourished people in the developing world; 2) treating people afflicted with cow's milk allergies and gastrointestinal disorders, which is a significant segment in many populations of developed countries; and 3) filling the gastronomic needs of certain consumers, which is a growing market share in many developed countries [2].

Milk is a complete food for young animals and is consumed by humans due to its high nutritional value; all of its nutrients are good for human health. Milk, excluding water, contains complete nutrients that are a source of protein, lipids, carbohydrates, vitamins and minerals. It also contains several bioactive compounds such as immunoglobulins, hormones, cytokines and nucleotides. On the other side, milk has been reported to contain the most common food allergens including β-lactoglobulin, α-lactalbumin and caseins. Several technologies of milk processing such as heat treatment, enzymatic hydrolysis and fermentation by lactic acid bacteria (LAB) is one strategy to destroy or eliminate the allergens of milk. Research aimed at producing hypoallergenic milk is of interest for future developments [3].

Nowadays, milk and milk products are available worldwide, being one of the favorite foods consumed by all ages. Fermentation of milk using LAB which has generally recognized as safe (GRAS) status, undoubtedly produces good quality and safe milk products for human health. The use of bacterial species that are generally considered to be probiotic such as *Lactobacillus helveticus* in yogurt and cheese production are beneficial. *L. helveticus* BGRA43 has strong antimicrobial properties potential against various sporogenic and pathogenic bacteria. *L. helveticus* BGRA43 is also able to hydrolyze β-lactoglobulin and reduce its allergenicity which contributes to improved digestibility particularly in people allergic to cow's milk [4]. Several *L. helveticus* species display specific probiotic properties such as the production of Angiotensin-I-converting enzyme (ACE; peptidyl-dipeptide hydrolase, EC 3.4.15.1) inhibitory peptides [5]. Milk is potential raw material which can be transformed to milk products with functional properties.

3.2 Milk Production and Quality

Milk is a highly nutritious food that meets the complete nutritional needs of humans of all ages. The consumption of milk either as milk per se or milk products varies considerably among regions, depending on tradition, availability, price and other reasons. World milk production across many countries has increased from decade to decade (Table 3.1). Most likely this is due to an increasing demand for milk because of population growth, urbanization, increased income per capita and changing food consumption preferences. It is interesting that poduction of milk in India is in the top ranked position. The contribution of goat and sheep milk production in India is high and plays a significant role in the national economy [6]. The importance of milk consumption in India is evident within long historical traditions of both urban and rural milk consumption, largely influenced by cultural factors.

Milk quality is very much dependent on several factors, particularly the type of animal (Table 3.2) and the management of the diet. The quality of milk is usually judged by its chemical composition (Figure 3.1) as well as its flavour and colour. The higher the nutritional content of milk the better the quality of milk. From a consumer's point of view, quality factors associated with milk are appearance, colour, and sensory attributes such as aroma and flavour. The colour of milk is perceived by the consumer to be indicative of purity and richness [7].

Differences in the chemical composition of milk of different animals will affect the quality of milk products. The quality of milk products produced using milk with different chemical composition will vary depending on the type of milk used. For example, buffalo milk contains a high percentage of fat which produces a better quality of yoghurt that is rich and creamy with an excellent mouthfeel compared with yoghurt manufactured from milk containing a low level of fat.

3.3.1 Effect of Animal Diet on Milk Productivity

Good feeding practice is necessary for dairy cows. Underfeeding of dairy cows will reduce milk productivity. Milk production and quality is strongly affected by the type of diet consumed by the

Table 3.1 Milk production across countries.

Country	1970	1980	1990	1996	1997	2000	2005	2010
	(Million tonnes)							
India	20.80	31.56	53.68	63.36	70.88	79.66	95.62	117.0
United States of America	53.07	58.24	67.01	69.86	70.80	76.02	80.25	87.46
China	1.96	2.93	7.04	10.19	10.09	12.37	32.02	41.14
Pakistan	7.45	9.01	14.72	22.97	23.58	25.57	29.44	35.49
Russian Federation	0.00	0.00	0.00	35.82	34.13	32.28	31.15	32.14
Brazil	7.42	12.06	15.08	19.20	19.36	20.53	25.53	31.82
Germany	28.18	32.10	31.34	28.80	28.72	28.35	28.49	29.67
France	22.85	27.89	26.81	25.82	25.65	25.74	25.71	24.21
New Zealand	5.99	6.70	7.51	10.01	11.06	12.24	14.64	17.01
United Kingdom	12.97	15.97	15.25	14.81	14.84	14.49	14.47	13.96
Poland	14.96	16.49	15.84	11.70	12.12	11.89	11.95	12.30
Netherlands	8.24	11.79	11.23	11.01	10.92	11.16	10.85	11.65
Mexico	4.11	7.23	6.27	7.71	7.97	9.44	10.03	10.84
Argentina	4.19	5.31	6.28	9.14	9.37	10.12	9.91	10.50
Australia	7.76	5.57	6.46	8.99	9.32	10.85	10.13	9.02
Canada	8.31	7.41	7.98	7.89	8.10	8.16	7.81	8.24
Ireland	3.08	4.72	5.40	5.30	5.26	5.16	5.38	5.24
Romania	3.12	4.34	3.81	5.07	5.01	4.62	5.55	5.06
Denmark	4.48	5.12	4.74	4.70	4.63	4.72	4.58	4.91

Table 3.1 (*cont.*)

Country	1970	1980	1990	1996	1997	2000	2005	2010
	(Million tonnes)							
Switzerland	3.20	3.68	3.88	3.88	3.88	3.91	3.96	4.11
Bangladesh	1.07	1.16	1.59	2.09	2.15	2.14	2.62	3.40
Austria	3.36	3.45	3.37	3.05	3.11	3.36	3.14	3.29
South Africa	2.91	2.50	2.48	2.64	2.85	2.54	2.87	3.23
Sweden	2.93	3.47	3.51	3.26	3.28	3.35	3.21	2.92
Chile	1.12	1.12	1.39	1.94	2.07	2.00	2.31	2.54
Finland	3.31	3.28	2.82	2.43	2.46	2.45	2.43	2.35
Afghanistan	0.75	0.84	0.83	1.56	1.72	1.66	1.73	1.82
Nepal	0.63	0.75	0.92	1.03	1.08	1.17	1.35	1.58
Norway	1.73	1.97	1.99	1.93	1.91	1.74	1.59	1.58
Indonesia	0.17	0.25	0.60	0.75	0.74	0.79	0.85	1.32
Thailand	0.00	0.03	0.13	0.34	0.39	0.52	0.89	0.85
Mauritania	0.24	0.23	0.27	0.30	0.30	0.32	0.37	0.39
Vietnam	0.02	0.04	0.06	0.07	0.06	0.10	0.23	0.34
Sri Lanka	0.14	0.24	0.25	0.29	0.29	0.16	0.17	0.21
World	391.82	465.66	542.47	547.02	550.77	578.88	648.00	720.98

Source: National Dairy Development Board (2013)

Table 3.2 Composition of milk (%) from different species.

Species	Fat	Protein	Lactose	Minerals	Total solids
Bison	1.7	4.8	5.7	0.96	13.2
Buffalo	10.4	5.9	4.3	0.80	21.5
Camel	4.9	3.7	5.1	0.70	14.4
Cow (Holstein)	3.5	3.1	4.9	0.70	12.2
Cow (Guernsey)	5.0	3.8	4.9	0.70	14.4
Goat	3.5	3.1	4.6	0.79	12.0
Horse	1.6	2.7	6.1	0.51	11.0
Human	4.5	1.1	6.8	0.20	12.6
Reindeer	22.5	10.3	2.5	1.40	36.7
Sheep	5.3	5.5	4.6	0.90	16.3

Source: McSweeney (2007)

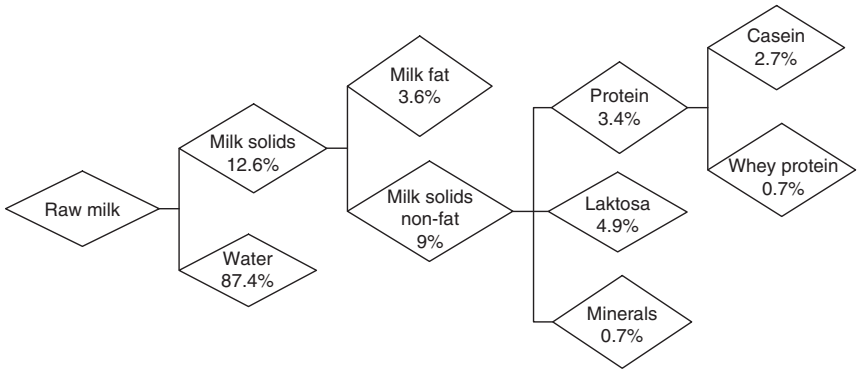

Figure 3.1 Gross composition of raw milk (Chandan, 2008).

animal. The proportion of forage and concentrate can influence milk composition. Of all milk components, milk fat is most influenced by dietary manipulations. Most of changes in milk composition due to dietary manipulation are related to changes in ruminal

acetate:propionate ratio. Feeding an imbalanced diet such as low energy:protein ratio may reduce milk fat and protein percentages. Forage is usually consumed without restriction while the amount of concentrate that should be enough for any particular cow depends on stage of lactation. A high concentrate diet tends to lower the milk fat percentage, since it depresses ruminal production of acetate and butyrate as precursors of milk fatty acid synthesis in the mammary gland. Feeding finely chopped forages also has a negative impact on milk fat production, because rumen pH will drop as the cow produces less saliva. Low rumen pH is not suitable for the activity of cellulolytic bacteria and depresses production of acetate and butyrate as precursors of short-chain fatty acid synthesis in the mammary gland. The use of legumes as substitutions for grasses may have a positive impact. Legumes are cheap and widely available in Mediterranean countries and are suitable for sheep and goat nutrition. This alternative feed resource contains secondary compounds, such as tannins. Tannin-containing feeds tend to increase milk yield and protein content, probably because they protect dietary proteins from ruminal degradation [8].

The use of probiotics as feed additive has been reviewed by [9]. The role of probiotics, either bacteria or yeast cultures, showed a positive effect on milk production. The role of bacteria probiotics include reduction of the incidence of diarrhea in calves and maintenance of intestinal health; prevention of rumen acidosis; controlled growth of pathogens in the rumen; production of conjugated linoleic acids (CLA), while the yeast culture includes an increase in the rate of establishment of cellulolytic population in the rumen; stabilisation of rumen pH; improvement of fiber degradation in the rumen; reduction of pathogen load; increased milk yield and increased total bacteria. Addition of dextran, a glucose polymer which is claimed as prebiotics, improved milk production of Holstein dairy cows significantly [10]. Milk fat from pasture fed cows seems to be higher in linolenic acid than milk fat from cows receiving preserved grass or maize. Indirect comparisons show that milk fat from maize silage diets is richer in short-chain fatty acid and linoleic acid when compared to grass silage diets. Compared to fresh grass, grass silage favors myristic and palmitic acids at the expense of mono- and polyunsaturated fatty acids, including CLA [11].

3.2.2 Organic Milk

Organic milk production is based on organic principles and objectives including naturalness and the recycling of nutrients [12]. Consumer interest in organic milk has been growing recently. The boost in organic milk sales is part of a wider growing interest in organic products, which resulted in an average annual growth rate of retail sales of organic food of nearly 18 percent between 1998 and 2005 [13]. However, the consumption of organic milk is still controversial. People may turn to organic milk for health benefit purposes, or environmental and animal rights issues. So far, when evaluating the health claims research does not support a health advantage of organic over conventional milk for any segment of the population [14]. The current meta-analysis using the Hedges' effect size technique, shows that organic dairy products contain significantly higher protein, α-linolenic acid (ALA), total omega-3 fatty acid, cis-9,trans-11 conjugated linoleic acid, trans-11 vaccenic acid, eicosapentanoic acid, and docosapentanoic acid than those of conventional types. It is also observed that organic dairy products have a significantly higher Omega3:Omega6 ratio and 9-desaturase index than the conventional products. The difference in feeding regime between conventional and organic dairy production is suspected to be the reason behind this evidence [15]. Based on a survey of processed milk from different UK retail outlets, raw milk at the farm level has higher concentrations of nutritionally desirable fatty acids and n-3:n-6 ratios in milk from organic production systems [16].

3.3 Types of Milk Products

Milk products have been widely produced and represent about 20% of the total economic value of fermented food all around the world [17]. Several milk products are representative of certain regions. Most of them are produced by fermentation technology, which is one of the oldest technologies of milk preservation which has been used for thousands of years. The market share of milk products, particularly fermented milk using LAB continues to grow. Nowadays, milk products are processed in large, highly mechanized and automated factories. The resulting milk products are distributed through large wholesale and retail outlets available to the consumers.

3.3.1 Liquid Milk as Beverage

The most widely available milk for human consumption is in the liquid form. Raw milk is milk that is collected directly from the animals. It is fresh, whole and natural as untreated liquid milk. Whole milk is suitable only for infants and young children under the age of five years because of its high fat and calcium content. The original taste of fresh milk is slightly sweet and salty from lactose and milk salts, with a delicate flavour from many odorous compounds. Natural milk provides nutrients not only for humans but also for microorganisms. Goat's milk has also been known for its beneficial and therapeutic effects on people who are allergic to cow's milk [18]. The presence of pathogens in raw cow's milk is estimated for *Campylobacter, Salmonella*, human pathogenic verocytotoxigenic, *E. coli* and *Listeria monocytogenes*, based on data indicating their occurrence in raw milk or on dairy cattle farms. The risks associated with raw milk consumption are mainly of a microbiological nature, and raw cow's milk does not really pose any risks at the nutritional level. Consumption of raw milk poses a realistic health threat due to a possible contamination with human pathogens. It is therefore strongly recommended that milk should be heated before consumption. With the exception of an altered organoleptic profile, heating, in particular ultra-high temperature and similar treatments, will not substantially change the nutritional value of raw milk or other benefits associated with raw milk consumption [19].

The most common process applied to liquid milk to fight against spoiling microorganisms and prolong its shelf life is pasteurization. Pasteurization is a process of heat-treating every particle of milk to remove harmful organisms and allows the prolonged shelf life of milk. The process operates under a certain temperature and is held continuously at or above the given temperature for a specified time. The range of temperature is 63°C up to 138°C for ultra pasteurization. There is also a need to ensure that commercial pasteurization processes and equipment are controlled and maintained so that milk is subjected to the correct time or temperature treatment in the holding tube [20]. The official US government definition of an ultra-pasteurized dairy product stipulates 'such product shall have been thermally processed at or above 138°C for at least 2 seconds, either before or after packaging, so as to produce a product which has an extended shelf life'. Also known as

the high-temperature–short-time (HTST) process, it has been effectively used for decades. However, HTST pasteurization can affect the aerobic plate count present in processed milk during refrigerated storage. The endospore-forming psychrotolerant bacteria present in milk grow more effectively in pasteurized milk after a higher heat treatment [21]. Microfiltration represents one possible processing tool for removal of bacterial spores, with the goal of extending milk shelf life [22].

There are strategies to extend the shelf life of milk driven by an interest to produce fresh, nutritious and safe products by non-thermal processing. Pulsed electric fields (PEF) treatments, having a peak electric field of 35 kV/cm and 2.3 μs of pulse width, at a temperature of 65°C for less than 10 s, were applied to produce an extended-shelf-life product. PEF applied immediately after HTST pasteurization extended the milk's shelf life to 60 d, while PEF-processing after eight days caused a shelf life extension of 78 d [23]. A high pressure N_2O process can be used as a low temperature alternative process for milk microbial stabilization. It is a new low temperature method to milk pasteurization. In this regard it is worth noting that the treatment time needed to reach a complete microbial inactivation depends on the reactor design, and could be minimized by investigating and optimizing the contact between N_2O and the sample. This would increase the kinetics of dissolution of the gas into the liquid phase. To be exploited on an industrial scale, further studies are needed to confirm the low-impact of N_2O process towards sensorial/chemical features of the treated product [24]. On a rural co-operative milk industrial scale, more appropriate technology is needed. A solar-powered icemaker has been applied to preserve milk and milk products in rural areas of Kenya which is energy efficient and sustainable [25].

3.3.1.1 Skim Milk

Many people prefer low-fat milk, since it is more appealing and a good compromise. Skim milk has as much fat removed as possible and has about half the calories of whole milk. It is the best choice for adults, and is the only type of milk that should be consumed by people on strict low-fat diets. Unfortunately, skim milk has a very 'thin' flavour and an unappealing bluish cast. Skim or non-fat milk contains less than 0.5% milk fat and not less than 8.25% solids-not-fat [26].

3.3.2 Cream

Cream is the fatty part of milk, and creams of different fat contents can be prepared by the separation of milk fat from the nonfat solids portion of milk. Cream is a richly flavoured product, which makes it desirable for use in applications such as desserts, cakes, and some chocolate confectionery [27]. The fat content of cream products varies from about 10–50%. Milk fat globules can be affected greatly by processes applied to the milk, particularly homogenization, which has significant implications not only for the properties of milk fat globules, but also for casein micelles in milk [28]. Internationally, cream products are not yet standardized by law. However, there are a range of products that consist of low fat content from milk cream, such as 'coffee cream' (≥10% fat, Germany), 'half-and-half cream' (≥10.5% fat, USA), 'half cream' (≥12% fat, UK) or 'light cream' (≥12% fat, France). Traditional whipping cream has 30 to 40% fat, whereas double cream contains about 50% fat. Creams of high fat content are also essential ingredients in dairy or non-dairy products such as some fresh cheese varieties or cream liqueurs. Butter is manufactured from cream (30–80% fat) by phase inversion [29].

3.3.3 Butter

According to the Codex Alimentarius Commission, butter is defined as a fatty product derived exclusively from milk. A 100 g portion of butter must contain a minimum of 80 g fat and a maximum of 16 g water and 2 g nonfat milk solids. A similar definition is used within the EU as stated in Council Regulation No. 2991/94 regarding standards for spreadable fats, such as butter, blends, and spreads [30]. Butter is a water-in-oil emulsion and essentially the fat of the milk. The main constituents of a normal salted butter are fat (80–82%), water (16–18%), salt (ca. 1%) and protein (ca. 1%). In addition, butter contains fat-soluble vitamins A, D, and E [31]. Butter is generally made from cream by churning and working. It contains 80% fat, which is partly crystallized. The churning process is most easily done at a temperature of around 15 to 20°C. Therefore, butter typically is a product originating from regions having a temperate climate. In addition to accumulated practical experience, a good deal of science has now been incorporated in butter making, enhancing the shelf life and quality of the product and the economy of manufacture [32]. Butter is produced by a mechanical phase inversion

of cream, an oil-in-water emulsion, to reach a water-in-oil emulsion. More precisely, butter consists of a continuous fat phase in which water droplets, fat globules and a network of fat crystals are dispersed [33].

3.3.4 Ice Cream

Ice cream is a frozen confection made from milk, sugar, stabilisers such as carboxymethylcellulose, emulsifiers such as polysorbate and flavouring [34]. Milk fat content may vary with respect to region and legislation, although the products can be modified as low fat ice cream. Like other milk products, ice cream is a relatively innovative matrix for the application of probiotics. The incorporation of probiotic bacteria into an ice cream formulation must not affect the product's global quality. Therefore, the physical–chemical parameters involved in the quality control of this product, such as the melting rate, and the sensory features, ought to be the same or even better, when compared to a conventional ice cream. The preparation procedure is as usual and probiotic cultures were added as adjunct cultures during the process [35].

The enrichment of ice cream with dietary fibres is an effective way to enhance nutritional and physiological aspects and to promote functionality by influencing rheological and thermal properties of the final product. The addition of dietary fibres contributes to the modification and improvement of the texture, as shown by their microstructure (Figure 3.2), sensory characteristics and product shelf life.

Parvar and Goff (2013) used basil seed gum (BSG) as a novel source of hydrocolloid to stabilize ice cream. BSG was compared to a commercial blend of carboxymethyl cellulose, guar gums and control. There was no significant difference in ice crystal size after hardening, but the presence of BSG reduced ice recrystallization compared to commercial gums and the control. The addition of BSG reduced the rate of ice crystal growth by 30–40% compared to the commercially stabilized ice creams. BSG also decreased the meltdown rate and increased the particle size, thus suggesting that BSG produced a different structure compared to the controls, possibly by lowering the air and fat interfacial tensions [36].

Many studies have focused on the production of probiotic ice cream using cow's milk. The incorporation of probiotic bacteria

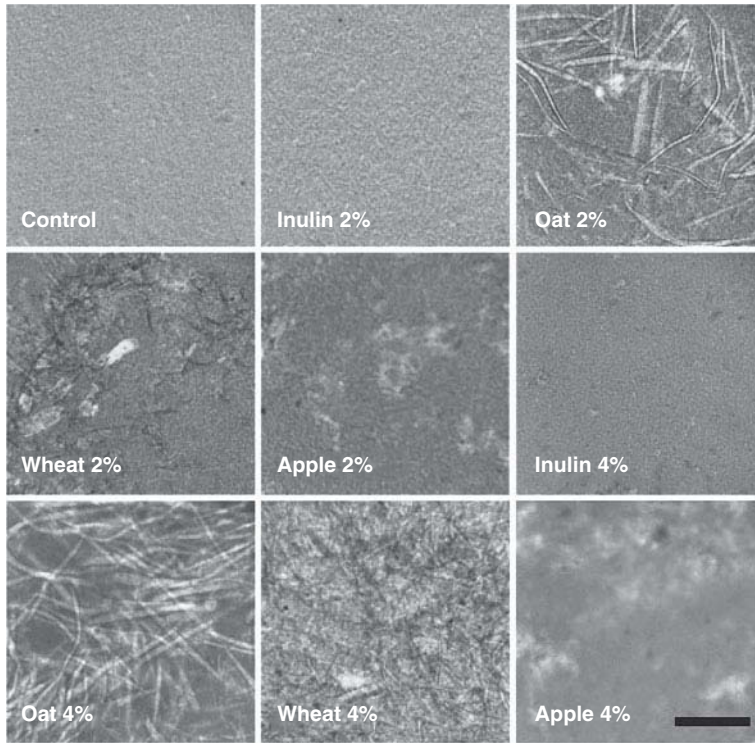

Figure 3.2 Light micrographs of aged ice cream mixes with or without addition of dietary fibre taken at room temperature with a magnification of 40× (scale bar 50 μm) (Source: Soukoulis *et al*, 2009).

into different types of ice cream is also highly advantageous for providing a food rich in health benefits. Supplementation of probiotic bacteria has been found to have little effect on its flavour, texture or compositional characteristics. Goat's milk may be considered a viable alternative to cow's milk in manufacturing probiotic dairy desserts with high probiotic viability. It is suggested that frozen storage is unlikely to produce any adverse effects, and may in fact facilitate improvement in the organoleptic characteristics of probiotic goat's milk ice cream over extended storage periods [37]. Ice cream can be supplemented with prebiotics such as inulin and oligofructose to improve probiotic stability as well as the sensory and physicochemical characteristics of synbiotic ice cream [38].

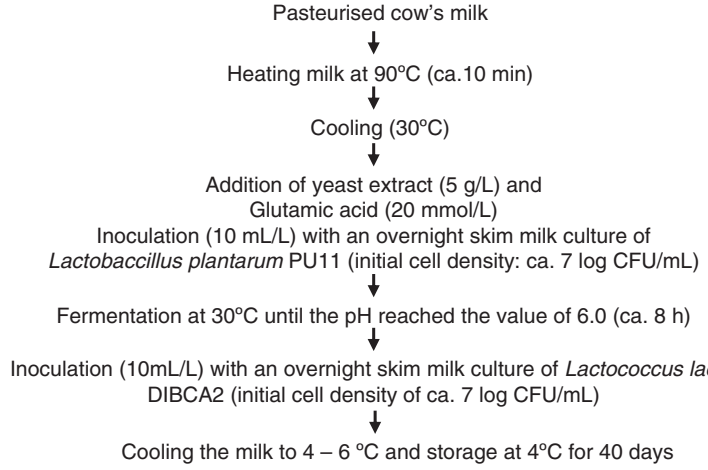

Figure 3.3 Protocol for the manufacture of the functional fermented milk started with *Lactobacillus plantarum* PU11 and *Lactococcus lactis* DIBCA2. (Nejati *et al*. 2013)

3.3.5 Fermented Milk Product

Fermented milks have been reported as milk products with health benefit properties for many years. By fermentation using LAB the main components of milk, i.e., lactose, protein and fat, contribute greatly to the functional properties of milk products. Milk lactose is fermented to lactic acid and it improves the physical properties of casein and thus promotes digestibility. It also improves the utilisation of calcium and other minerals. Milk protein is degraded to some free amino acids and bioactive peptides. Bioactive peptides are a supplement to functional foods. Milk proteins are the main source of biologically active peptides. The role of bioactive peptides includes anti-microbial activity, blood pressure regulation, and mineral or vitamin binding. Milk fat is also degraded by fermentation.

Among bioactive peptides, ACE inhibitory peptides are widely studied. Another biogenic compound of interest is γ-amino butyric acid (GABA), an ubiquitous non-protein amino acid involved in neurotransmission. A functional fermented milk using *Lactococcus lactis* DIBCA2 and *Lactobacillus plantarum* PU1 was produced (Figure 3.3) that may have potential application for the management of mild hypertension. The product had an ACE inhibitory activity (IC_{50}) of 5±2 µg/ml and GABA ca. 77.4 mg/kg after 120 h of fermentation [39].

3.3.5.1 Yoghurt

Yoghurt is a milk product fermented by LAB. It represents the most popular milk product worldwide. Yoghurt is produced from preheated milk with the addition of materials such as flavourings or colourants that make it more attractive than other milk products to consumers. Figure 3.4 is a schematic illustration showing the different processes for the manufacture of yoghurt-related products [40]. Nowadays, the health promoting properties of yoghurt are widely produced and well accepted by consumers. A recent study on adult Americans concluded that yoghurt consumption was significantly associated with lower levels of circulating triglycerides, glucose, and lower systolic blood pressure and insulin resistance.

Yogurt is a good source of several micronutrients and may help to improve diet quality and maintain metabolic well-being as part of a healthy, energy-balanced dietary pattern. The micronutrient composition in yoghurt is more concentrated than in milk [41]. It is also possible to produce a yoghurt-type milk product with desirable organoleptic properties, by combining the particular nutritive value of goat's milk with potential functional properties derived from the selected multi-strain culture of LAB [42].

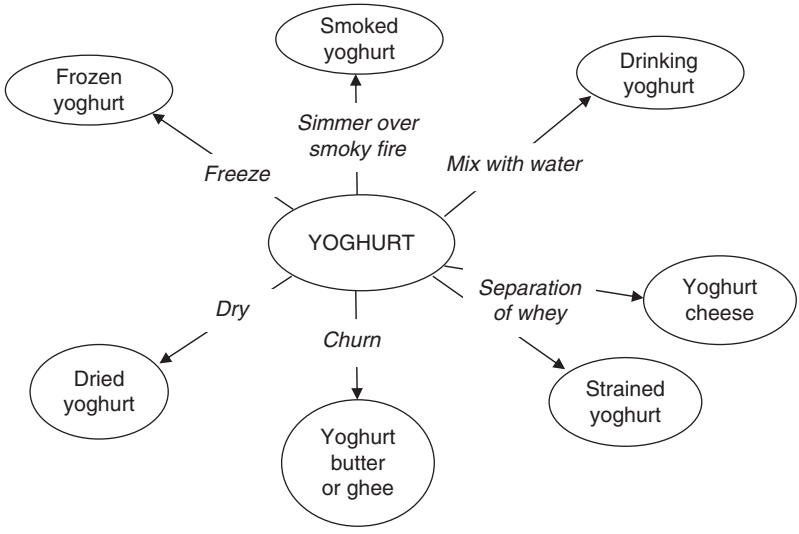

Figure 3.4 Schematic illustration showing the different processes for the manufacture of yoghurt-related products (Tamime and Robinson, 2000).

New processing methods such as high-pressure processing are of interest for their potential to achieve specific and/or novel functionalities and/or improved efficiencies, including reduced chemical and water use. An investigation of the use of simultaneous pressurization and heating of milk before the manufacture of stirred yoghurt reported that yoghurt milk treated by high pressure imparts significantly improved functional properties to the final yoghurt [43].

3.3.5.2 Cheese

Cheese making is an art of science and technology to convert raw materials with the action of selected microorganisms to produce cheese, a highly nutritious food. Milk is the raw material and main ingredient in cheese manufacturing. Based on the properties of milk, cheese making was originally carried out with the main purpose to extend the shelf life and conserve the nutritious component of milk. However, besides the purpose to preserve the milk, recently there is a growing interest to develop a variety of cheese products with other advantages, such as improving and diversifying the taste or flavour of cheese products to fit the consumer's choice. It has been widely accepted that flavour represents product characteristics of cheese.

The principle of cheese making is lactic acid fermentation, starting with production of lactic acid from lactose. Moreover the cheese making process is complex and consists of many steps, which involve the chemistry and biochemistry of milk, microbiology and enzymology. The contribution of microorganisms is essential to all cheese varieties and plays an important role during both the manufacturing and ripening of cheese. Cheese cannot be made without the presence of certain microbial species, and in most cases LAB is a necessity. Several LAB are widely used and their role can be divided into starters and non-starters or adjunct cultures as secondary microorganisms. Starter cultures can be either mesophilic or thermophilic LAB with their optimal growth temperature at about 30 and 45°C, respectively. Interest in using thermophilic isolates of LAB is because they are good acid producers, therefore suitable as starter cultures. Starter cultures involved in production of acid during manufacturing also contribute to the ripening process. Adjunct cultures are not responsible for the production of acid, but contribute more during the ripening process. It is during the ripening

process that flavour formation and the characteristic flavours of individual cheese varieties develop. Improvement of Cheddar-type cheese with addition of green tea extract (GTE) was reported. Total protein, fat, micellar calcium content, and yield of cheese were not significantly affected by GTE addition. Enrichment with GTE showed higher hardness and a loss of cohesiveness and springiness and significantly affected the colour of the cheese. The addition of GTE at a concentration of 1 or 2 g/kg of milk significantly increased the antiradical activity of cheese by 25 and 44%, respectively. GTE enrichment of Cheddar-type cheese could result in new products with increased health benefits [44]. Low-fat cheeses made with functional ingredients or additives, such as fat replacers, are being consumed by people who are interested in healthy and tasty foods. Fat replacers are used in the low-fat cheese-making process because those particles improve the texture and the nutritional value of low-fat food products [45]. Fat replacers can be used to improve the sensory and functional properties of reduced-fat cheeses. Cheesemilk for cheeses was supplemented with gum tragacanth (GT) at a level of 0.05% (w/v), and the cheeses were ripened at 8°C for 10 months. GT can have a major effect on many of the rheological, sensory and functional properties of Cheddar cheese. The major effect of GT appears to be its ability to alter the composition and indirectly lower the pH of cheeses. These results suggest that GT appears more suited to enhancing the textural and functional properties of half-fat Cheddar cheese than its sensory properties [46].

3.4 Conclusion

Milk and milk products represent an important food for humans as they provide valuable nutrients for all ages. Research on developing new milk products has been widely carried out with the application of new technologies, and the products can be categorized as functional foods.

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Processing and Preservation of Meat, Poultry and Seafood

Elisabete M.C. Alexandre*, Cristina L.M. Silva and Teresa R.S. Brandão

CBQF – Center for Biotechnology and Fine Chemistry – State Associated Laboratory, College of Biotechnology, Catholic University of Portugal/Porto, Porto, Portugal

Abstract

Contamination of animal carcasses and raw products by microorganisms, including spoilage and pathogenic types, is practically unavoidable. Additionally, muscle foods such as meat, poultry and seafood are excellent food matrix for microorganism development due to their high moisture content and diversity of nutrients. Moreover, all foods are subject to physical, chemical and biological undesirable deterioration during storage. So, to extend products shelf life and to assure their safety from a microbiological point of view, it is necessary to apply adequate decontamination processes. However, the quality characteristics of the processed final products may be affected. To inactivate undesirable microorganisms and enzymes responsible for quality decay, adequate treatments should be applied. These are essentially based in high- temperature and low-temperature conditions, moisture control, radiation technologies, chemical preservatives addition and microbial antagonisms.

This chapter provides an overview of physical, chemical and microbiological methods frequently used to preserve muscle foods, focusing on safety aspects and quality characteristics of processed foods. Hurdle combinations of methods and modified atmosphere packaging that allow shelf life extension are also highlighted.

Keywords: Thermal treatments, drying, cooling, curing, smoking, chemical compounds, radiation, food safety and quality

**Corresponding author*: elisabete.alexandre.pt@gmail.com

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4.1 Introduction

In general, meat is defined as the flesh of animals consumed as food, which is mostly the muscle tissue of an animal. For centuries, meat, poultry, seafood and their derived products have constituted some of the most important foods consumed worldwide. The human body has complex nutritional requirements that must be fulfilled and those food products are one of the major important sources of a wide variety of essential nutrients in human diet. The animal muscle is typically composed of 60–80% water, 18–20% protein, 0.5–19% lipids, 1–1.5% minerals and a trace of carbohydrate [1–3]. However, this composition varies extremely, mainly in the lipid content (0.5–19%), which in turn affects the amount of water present in the tissues. Animal characteristics (e.g., species, breed, age, gender and weight), nutritional regime (type of feed and feeding), environmental conditions and geographical factors, hygienic practices and disease control programs, may affect meat characteristics [3].

The high protein content is one of the most important characteristics of meat. It plays an important role in the human diet as a source of essential amino acids, such as leucine, lysine, threonine, methionine and tryptophan required for cellular maintenance, growth, and functioning of the human body [1, 4, 5].

Nowadays, fish is more recognized as a supplier of micronutrients, minerals and essential fatty acids, than for its protein value. Vitamins A and D, calcium, phosphorus, magnesium, iron, zinc, selenium, fluorine and iodine are some examples of the essential micronutrients and minerals for the human diet that are present in fish [6].

Once animals' muscles are nutrient enriched matrixes, they provide a suitable environment for proliferation of spoilage microorganisms, becoming muscle foods, one of the major sources of pathogens that may cause foodborne diseases in humans. Food safety is a priority topic for authorities and consumers worldwide. Therefore adequate preservation processes must be applied in order to assure its safety and quality. The application of methods and technologies to foods that alter their raw state and characteristics is designated by *food processing*. Food processing has three major goals: to make food safe while providing products with the highest quality attributes, to make food into forms that are more convenient or more appellative to be consumed, and to

extend shelf life [7]. Temperature plays an important role in food processing: high temperatures are crucial for microbial death or inactivation (safety point of view), whereas low temperatures are often applied for long-term food preservation, preventing microbial growth and retarding reactions of quality alterations, in a joint perspective of safety and quality.

Food processing dates back to ancient ages. Foods were sun dried, fermented, salted, smoked and frozen in glacier waters aiming at longer preservation. Alterations in food taste, texture and appearance caused by processing were later found to be also appealing. Food processing technologies had a great development after World War II, with the expansion of a consumer society in developed countries. Processes such as spray drying, freeze drying and irradiation were innovations of that time, as well as the introduction of sweeteners, food colouring agents and preservatives such as sodium benzoate [1]. Over the past years, there has been a growing interest in the alteration and control of the atmospheres within food packages aiming at food preservation and shelf life extension. The development of technologies and related equipment were fundamental for the advance of food processing operations [8], namely cook-chill, vacuum packaging systems, ionizing irradiation, phage technology, high pressure, and hydrodynamic shockwave.

4.2 Food Quality Characteristics

Food quality is a broad concept, directly related with physical, chemical and sensorial characteristics of a food. However, quality is likewise associated with health and safety aspects. The term *healthy* is associated with food components that have a positive and functional impact on human health, while *safety* is related to the certification that undesirable and/or hazardous agents such as biological, chemical and physical entities are not present in fresh and processed foods [9].

In muscle foods, quality is affected by food composition, nutrients, additives, colourants, and flavourants and by the presence of spoilage microorganisms. The perception of quality can be linked directly to human senses such as vision (colour, moisture, amount of fat, overall appearance), touch (sliminess, elasticity, softness, hardness), mouthfeel (texture, softness, tenderness, juiciness,

flavour, chewy sensation), smell and taste (signs of deterioration and specific chemicals). These are important and define consumer acceptability [9, 10] . The most relevant are described as follows:

Colour is the prime characteristic that influences the initial selection of food by the consumer. Consumers prefer fresh red meats; the colour is related with high content of oxymyoglobin. High contents in metmyoglobin have a negative effect on red meats due to the development of brown colour. In poultry and seafood the skin colour is the main perceived characteristic. In these products the colour of muscles is relevant if the products are retailed and the skin removed [5, 11]. Defects, essentially cosmetic such as blood splash and staining of fat with blood from drip, can also have impact on the perception of quality. The **moisture** and amount of visible **fat** are also relevant [5, 10, 12].

Texture is considered to depend on the size of the bundles of fibres into which the perimysial septa of connective tissue divide the muscle longitudinally. Muscle foods have different textural characteristics due the nature of fibres, fluid/fat exudation, and connective tissue [4, 10]. The quality of muscle food is related to animal breed, diet, marbling, postmortem pH decline and aging [10]. No conscious sensation is derived from the digestion, when the amino acids, fatty acids, vitamins, minerals and other components are liberated and absolved into the body. However the organoleptic sensations may improve or prejudice the efficacy of the digestion process by their reflex action on the production of gastric and intestinal fluids [4].

Tenderness depends on type of muscle and post-mortem events concerning onset and resolution of rigor (tenderization). The phenomena involved in tenderness are mechanical (hardness, cohesiveness, elasticity), particulate (grittiness and fibrousness), and chemical (juiciness and oiliness). Although consumers routinely pay more for cuts of meat that are typically more tender, poultry breeds produced from slow-growing with high cereal diets are usually more desirable, due to their firmer meat.

Juiciness is related to water-holding capacity of meat and to marbling, also affecting consumer choice; dry meat or excessive drip and exudation are undesirable [5, 10, 12].

Flavour is a complex sensation that is related to odour, taste, texture, temperature and pH. Among these characteristics, odour is the most relevant one because without it the primary taste sensations (bitter, sweet, sour or saline) predominate. Flavour results

from volatile and nonvolatile compounds, but its perception varies in intensity from person to person [4, 13].

4.3 Deterioration and Microbial Contamination

All foods suffer physical, chemical and biological deterioration throughout time. The deterioration process may include loses in organoleptic desirability (colour, texture and flavour), nutritional content and aesthetic appearance derived from inappropriate storage temperature and humidity, presence of oxygen and/or light and physical stresses [14]. These conditions may allow: *(i)* microbial growth and activity, mainly bacteria, yeasts and moulds, *(ii)* activity of enzymes responsible for quality alterations and *(iii)* other deteriorative chemical reactions. Infestation by macroorganisms, such as insects, parasites and rodents affects the products' safety and compromises quality.

Muscle foods such as meat, poultry and seafood are excellent food matrix for microorganism development due to their high moisture content and diversity of nutrients. The microorganisms may spoil meats by infection of the living animal or through postmortem contamination. Living animals carry microorganisms on their external surfaces and in the gastrointestinal tract, which may serve as sources of microbial contamination for carcasses during the slaughtering, dressing, chilling, and cutting processes. So, carcass and meat contamination may happen through different vehicles such as air, water, feces, knives used during exsanguination and cutting, hides, fleece, feathers, the gastrointestinal tract through accidental spillage of its contents during evisceration, lymph nodes if inspected by incision or otherwise cut, contact with other contaminated carcasses, employees and through the processing environment and equipment [15, 16].

In theory, bacteria, yeasts and moulds may attack all food constituents. There are some microorganisms that ferment sugars and hydrolyzed starches and cellulose, others have the capacity to hydrolyze fats producing rancidity and others digest proteins and produce putrid and ammonia-like odours. Some other types form acids and make food sour, produce gas and make food foamy and form pigments discolouring the foods. Only a few microorganisms are responsible for the toxins production that results in food poisoning [14]. *Clostridium botulinum, Clostridium perfringens, Salmonella*

spp., Listeria monocytogenes, Campylobacter jejuni, Staphylococcus aureus, Shigella spp., Vibrio parahaemolyticus, Vibrio cholerae Ol, Bacillus cereus, Yersinia enterocolitica and *Escherichia coli* are some of the most important microorganisms responsible for human foodborne illness that may be associated with meat, poultry and seafood consumption. However, among the thousands of genera and species of microorganisms, not all cause diseases or food spoilage, some are even used to preserve foods [17–22].

Healthy uninfected muscle food has its own enzymes, whose activity is intensified after slaughter. Enzymatic reactions are delicately balanced in the normally functional living animal; however, this balance is affected when the animal is killed. For example, in the living animal, the pepsin present in the animal intestine helps in the protein digestion but it does not digest the intestine itself. However, after slaughter, pepsin contributes to proteolysis of the organs containing it. These enzymes should be inactivated aiming at meat preservation, otherwise they will continue catalyzing chemical reactions within the food, fostering deterioration [14].

4.4 Physical Methods of Preservation

There are some processing methods and technologies that can be applied to inactivate enzymes and microorganisms, preserving meat, poultry and seafood products longer. Physical methods are essentially used to remove soil and solid matter from the external surfaces of animals or carcasses and to reduce microbial loads. When the control or inhibition, irreversible inactivation or destruction, or mechanical remove of micoorganisms is performed without the use of antimicrobial additives or products of microbial metabolism they are generally called physical methods of decontamination [23]. Some examples of these methods are related with the washing/cleaning and/or hair trimming before slaughtering, dehairing and defeathering, knife trimming, and washing of carcasses. They are essentially based in high-temperature and low-temperature conditions, moisture control and radiation technologies.

4.4.1 Preliminary Processes

4.4.1.1 Seafood

Different types of gear and fishing methods will affect the biochemical state of the fish muscles. At the time of capture, the

fish should be calm; if necessary, they should be anaesthetized to reduce strain and simplify handling and killing. A careful and gentle handling will avoid physical damage and prevents enzymatic and bacterial activities. Dressing operations of the catch include heading, bleeding, and gutting. They should take place immediately after killing, without significant bacterial contamination of the flesh. The larger fish are sorted and gutted by hand or mechanically to remove digestive enzymes responsible for early autolytic changes and to prevent entry of nematodes from the intestines into the muscle tissues. Afterwards, they are washed and cooled. Rigor mortis commences earlier and it is essential to chill the fish (to temperatures below 0°C) or to freeze it as soon as possible after catch, preferably before onset of rigor mortis, for prolonged shelf life. Rapid chilling slows down the enzymatic and microbial activities. Antifreeze proteins (specific cryoprotectants) may be used to lower the freezing temperature, which improves the quality (flavour and texture) of frozen products [24–27]. Fish preservation onboard is traditionally done by bulking, shelving, and boxing. For the most part, fish are stowed in a bulk of ice, however, for high-value fishes boxing offers the best method for onboard storage [28].

Shellfish, both mussel and crustacea, should be kept alive until sold to the processors, because their muscle spoils very quickly after death.

4.4.1.2 Poultry

The typical operations involved in poultry processing are receiving and weighing, unloading, stunning, bleeding, scalding, feather removal, singeing, washing, feet removal, oil gland removal, venting, opening cuts, viscera drawing, inspection, giblet salvage, lung removal, head removal, crop and windpipe removal, washing, chilling, grading, cutting and packaging, distribution and further processing [19]. Scald tank, feather removal and evisceration are considered the major points of cross-contamination for *Salmonella* and *Campylobacter*. Mechanical processes for feather removal and viscera, and spray washings minimize cross-contamination and reduce microbial loads of carcasses. Combined physical and chemical treatments, such as heat and chemical solutions, are typically employed during processing to further reduce microbial contamination of carcasses [29]. The chilled immersion step can be a potential source of cross-contamination, however, when there is an

efficient control of temperature, water usage, pH and chorine levels, it is verified an effective control of pathogens.

4.4.1.3 Meat Trimming

When materials such as feces or ingesta contaminants are visually detectable on animal carcasses, it usually means that poor process sanitation has occurred. Properly performed knife trimming or mechanical removal of tissues related to gross contamination, have been proven to be effective in the elimination of microorganisms associated with visual foreign materials. A well-implemented knife trimming may be advantageous when compared to mechanical removals, such as spray washing/decontaminating processes at ambient temperature water. In the trimming operation, a physical removal of tissue is performed, avoiding or reducing the opportunities for microorganism translocation to non-contaminated adjacent areas [23, 30]. However, some researchers suggest that knife-trimming may not be economically viable because of the amount of product waste [31, 32].

Some slaughter plants use warm (~42°C) 2% lactic acid on line spray cabinets and others have adopted spray washing with hot water at 74°C. It was suggested that the use of hot water may be more effective than warm lactic acid, but the acidified environment may result in further microbial reductions during refrigerated storage [23, 30].

The impact of the trimming process in microbiological loads is restricted only to those microorganisms associated with surfaces trimmed, and it has been suggested that trimming, as a whole, may have a negligible impact on the overall hygienic condition of meat [23].

4.4.2 Water Spray-Washings

The majority of spray-washing systems are automated, allowing similar exposition to spray-washing treatment of all carcasses. The success of this operation may depend upon the tissues condition and degree of bacterial attachment. In a first stage, bacteria attachment to tissues is a reversible attraction that results from van der Waals forces. Increasing the contact time induces the occurrence of stronger and irreversible bindings involving exopolymer (polysaccharide) production and glycocalyx development, and subsequent

biofilm formation of a micro-colony. Consequently, water spray-washings should be performed as quickly as possible and, preferentially, close to the contamination source. The performance of spray-washings can be improved by optimizing the type and configuration of the equipment, increasing the number of nozzles, changing water temperature and pressure, optimizing target surface distances and exposure times [23].

4.4.3 Control of Temperature

4.4.3.1 Thermal Processing

The application of high temperature conditions is the most commonly used method for food preservation. The main objective of thermally-based treatments is to guarantee the destruction of pathogens and spoilage microorganisms and inactivate enzyme, which minimize spoilage reactions and proliferation of undesirable microorganisms. When a food is cooked, only partial elimination of hazardous and spoilage microorganisms and enzymes may occur. To attain a safe product with extended shelf life it is necessary to destroy all pathogenic microorganisms and their spores that can grow and/or produce toxins in order to reduce spoilage microorganisms. This is only achieved with more severe treatments. However, there should always be a balance between food processing conditions that allow safety from a microbiological perspective, and the negative impact of temperature on organoleptic and physical properties of the products [5, 33].

Heat penetration in foods depends on their structure, composition, physicochemical characteristics, heat transfer properties of the package or container and heating medium. Heat distribution within the product essentially depends on thermal properties of the food itself (thermal conductivity and specific heat) and physical properties (food density and geometry). In meat products subjected to thermal processing, heat and mass transfer phenomena occurs simultaneously. Biological and biochemical changes occur: microbial cells become unable to reproduce and enzymes are inactivated (protein unfolding) [1, 33].

Thermally-based processes may involve different heating media: *(i)* hot air (heat transfer is only moderate, but it can increase through air humidification); *(ii)* steam (heat transfer is higher due to steam condensation); *(iii)* hot water (heat transfer is relatively good);

(iv) hot fat or oil (very good heat transfer properties); *(v)* radiant heating (heat transfer is very good and high surface temperatures are attained); *(vi)* dielectric heating (microwave and radio frequency radiation, which does not depend on heat transfer through a surface); *(vii)* extrusion (this process cannot be applied to conventional meat products but it is used to obtain a new type of restructured meats) [5].

Thermal treatments can be generally divided into scalding, cooking, pasteurization and sterilization according to treatment severity [33].

Scalding treatments (temperatures around 65°C) are applied before freezing or drying processes, mainly with the purpose of inactivating enzymes. These treatments enhance vacuum formation during canning or vacuum packaging by removing the gas trapped in the muscle fibres [33].

Cooking can be carried out in dry or moist conditions, or by a combination of both. Dry cooking includes roasting, broiling, frying and grilling, while moist cooking refers to stewing, braising, boiling and covered roasting. The temperatures involved are around 100°C, although frying implies the use of boiling oil at temperatures higher than 200°C. The main objective of a cooking process is to improve the palatability; however, chemical, physical and microbial changes occur also conferring a longer shelf life to cooked meats [1, 5].

Pasteurization eliminates pathogenic vegetative cells; nevertheless spores and heat-resistant microorganisms can survive. To minimize microbial surviving, this process is often used in combination with other methods, such as refrigeration, chemical additive addition, vacuum packaging and fermentation. Pasteurized products generally reach an internal temperature of 71°C, but the efficacy of the thermal treatment depends on the combination of temperature and exposure time. After treatment exposure of meat, tissues should be rapidly cooled in order to minimize the impact of heat on product colour [1, 23, 33].

Sterilization eliminates or kills all forms of microbial life, including spores and heat-resistant microorganisms. Commercially sterile foods are heated for different periods of time at a high temperature (up to 121°C), and *Clostridium botulinum*, *Clostridium sporogens* and *Bacillus stearothermophilus* are frequently used as indicators to assure the success of the sterilization procedure. Sterilized foods have a considerably extended shelf life, and the use of a subsequent

preservation technique is not necessary [1, 4, 33]. To destroy spoilage and toxigenic microorganisms while simultaneously maintaining acceptable overall meat quality, it is convenient to use high temperatures for short periods of time [1, 4, 14].

4.4.3.2 Cooling

The meat, poultry and seafood industries use low temperature methods for food preservation. In contrast to thermal treatments, which are applied to inactivate or eliminate microorganisms, cooling methods are used to slow down or limit microbial growth. Depending on the temperature ranges used, cooling may be referred to as chilling or freezing [34]. Figure 4.1 represents heat removal during a cooling process. The amount of heat removed during freezing is higher than the amount of heat removed during chilling for a given temperature change. Consequently, freezing takes longer than chilling if the same temperature difference and heat transfer coefficient are used. Eventually, after freezing, muscle meat goes into a subcooling stage [34].

Chilling is the most widely used and effective means of muscle foods short-term preservation. It is used at the slaughter plant immediately after slaughter, during transport and storage, and at retail display. Chilling preserves food without changing its form or state, by lowering its temperature above the freezing point, from

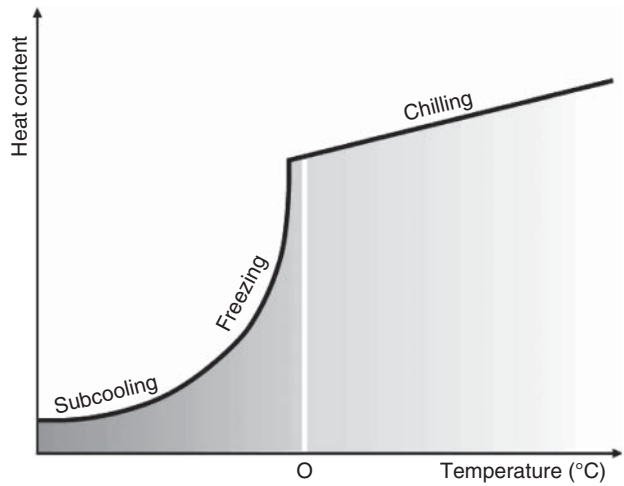

Figure 4.1 Typical variation of muscle meat heat content with temperature.

about 16 to –2°C. Pure water freezes at 0°C but most muscle foods do not begin to freeze till –2°C or lower temperatures are reached [14, 34].

Chilling and cold storage have slight adverse impacts on taste, nutritive content and other quality attributes of foods, for short-term storage periods. The temperature of the products should be lowered as fast as possible, to prevent microbial proliferation and to retain food quality. Conventional carcasses chilling may be carried out in a batch system or continuously. Both methods involve the use of a cool atmosphere, usually unsaturated cold air. The most important factors that affect the chilling rate of muscle foods are the air temperature and the air velocity over products surfaces. Although the size and weight of the carcass are also important, carcasses closer to the cooling fans are subjected to higher air velocities and lower temperatures [35]. Relative humidity is the most important parameter that influences mass losses; decreases in relative humidity induce higher mass losses.

Immersion systems using cold water or ice-water mixtures are commonly used in poultry industries. Spray chilling or accelerated chilling are other alternatives, with accelerated chilling being one of the most expensive operations in the meat processing industry [36].

Freezing is one of the oldest and most frequently used methods for long-term food preservation, in which the temperature of the product is reduced below its freezing point. A good frozen condition usually requires storage temperatures of –18°C or lower. When temperature is lowered below 0°C, there is a significant reduction of microbial activity and also a reduction in microbial loads; therefore, deterioration rates of foods decrease. The low temperatures also have a strong impact in the enzymatic activity and in oxidative reactions rates; this effect slows down deteriorative reactions, helping to avoid product deterioration. In addition, with ice crystal formation, less water is available to support deteriorative reactions and microbial viability [37, 38].

In general, frozen foods are synonymous with high quality products and only small changes of quality attributes occur when freezing and frozen storage are adequately and conveniently applied. Processing factors such as the freezing method, the freezing rate, the final temperature and frozen storage conditions influence the characteristics of final frozen products. Quality attributes such as texture, colour, flavour and nutritional content are mainly

related to the way ice crystals are formed [37]. Usually, when the animal tissue is cooled, the ice crystals are initially formed at the products surface and their growth strongly depends on the freezing rate. Some products require fast freezing rates (short freezing times) to assure the formation of many, but small, ice crystals within the product structure, avoiding cell shrinkage and reducing to a minimum the degree of freeze damage. In this case, small texture changes and small loss of nutrients will be verified through drip on thawing. Additionally, a rapid freezing followed by a slow thawing inactivates more microorganisms. Other products, due to their geometric configurations and sizes, do not allow a fast freezing. If the product is cooled slowly (high freezing time), large ice crystals will be formed, causing maximum disruption of tissue structure. Besides the freezing rates, the storage temperatures also play an important role in the frozen food quality. Fluctuations in the storage temperature may be harmful to product quality [27, 38, 39].

4.4.4 Control of Moisture

Drying is an ancient food preservation technique, which consists in the removal of water present in meat muscles by evaporation or sublimation. Primarily, the water moves by diffusion from the interior of food to the surface, where it is removed by evaporation. As a consequence, quality alterations occur: mass and volume of product decrease, affecting texture as well as aroma compounds. Due to the significant reductions of water activity, the microorganisms viability also decreases [40]. However, the drying conditions must be properly designed to attain maximum quality retention. When moisture is removed too rapidly from the food surface, a hardened layer is developed, which hinders the movement of the remaining water to the products surface. On the other hand, when water from the surface is not evaporated rapidly enough, it increases the possibility of the growth of undesirable microorganisms [1].

Fresh raw meat can be dried a few hours after slaughtering. Refrigerated and frozen meat must be properly thawed prior to further processing. Dehydration of meat often involves preparatory operations such as cutting of the carcass, trimming to remove undesirable material and cutting into strips or flat pieces. For some meat-based products, the meat must be chopped and mixed with other ingredients and seasonings [41]. Moreover, the rate of moisture

removal and of muscle fibre shrinkage is more rapid when products are precooked and processed further [1, 4].

Drying may be used as the only method for production of highly dehydrated and shelf-stable end products, or may be combined with other methods such as smoking, salting, seasoning, curing and ripening, leading to intermediate moisture products like ham and sausages. The combined treatments are used mainly to intensify the organoleptic characteristics (flavour and texture) and to improve palatability of the final products [41].

One of the most conventionally used techniques to dry foods is convective drying, based on heat of solar energy, microwave, or hot air stream. However, especially when heat is used in the drying process, the fat suffers oxidation and the production of off-flavours may occur [1].

Freeze dehydration is another drying technique, in which the water is removed by sublimation. The process consists of freezing the food and subjecting it to properly controlled vacuum and temperature conditions, allowing the water evaporation from the ice without ice melting. Microwave heating can be associated with this method to speed the process. After dehydration, proper packing must adequately protect the products. Freeze dehydration usually assures little or no shrinkage or distortion of food, as well as excellent texture and nutrient and flavour retention. However, the cost of this method is significantly higher compared to conventional ones, being at most five times more expensive [1].

4.4.5 Radiation Technologies

Electromagnetic radiation is a form of energy emitted and absorbed by charged particles (Figure 4.2). The types of electromagnetic radiation are broadly classified as lower energy (microwave, radio, TV) that occur as very long waves, intermediate energy (visible light, heat, and solar energy), high energy sufficient to ionize an atom (X-rays and γ-rays) and very high-energy radiation that occurs as radionuclide (uranium). Electromagnetic radiation is also commonly classified by the way it interacts with normal chemical matter in ionizing and non-ionizing radiation. Both radiations can be harmful to organisms, however ionizing radiation is far more harmful to living organisms per unit of energy deposited [42].

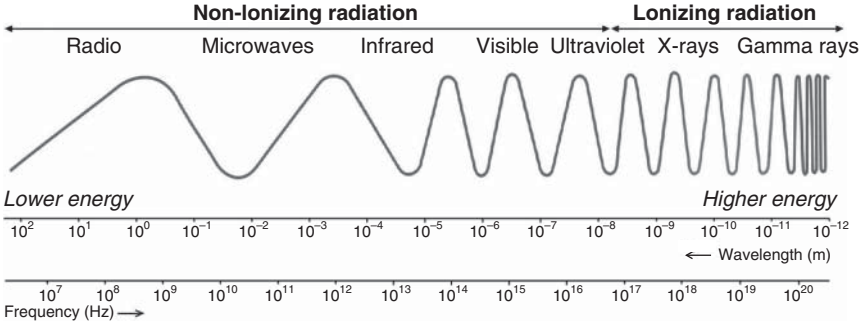

Figure 4.2 Electromagnetic spectrum.

4.4.5.1 Ionizing Radiation

Ionizing radiation has been applied as a preservation technique for meat since around 1940. In 1980, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) proposed that doses less than 10kGy should be accepted as a process for preserving all major categories of food [43]. Today this technology is approved in more than 55 countries worldwide for various applications and purposes. Ionizing radiation is one of the most effective physical technologies for inactivating pathogens in meat and in meat-based products, poultry and seafood. This technology can be implemented at room temperature or lower, attaining a microbial inactivation similar to the ones obtained with pasteurization or sterilization processes. However, nutritive values and physicochemical properties of food products are better preserved. Ionizing radiation also maintains the integrity of products without leaving chemical residues. Moreover, the foods can be processed after they have been sealed in their final packaging, which prevents further cross-contamination during post-processing handling [44–46].

According to Codex Alimentarius Commission 2003, there are only three sources of ionizing radiation that are authorized for commercial radiation of food processing. The approved sources included γ-rays emitted by the radionuclides Cesium-137 or Cobalt-60, machine generated X-ray sources operating at or below an energy level of 5 MeV and accelerated electron beams generated from machine sources operated at or below an energy level of 10 MeV [43, 45, 46]. Each of these sources has specific advantages

and disadvantages that are presented in Table 4.1. Accelerated electrons do not require isotopes, but they need high energy levels for an effective radiation penetration of 1–2 cm. This method is insufficient to decontaminate carcasses (although superficial contamination will be eradicated), but it can be applied to other thinner meats and meat products [47].

The effect of ionizing radiation on microorganisms is related primarily to the molecular bonds of the microbial DNA. However,

Table 4.1 Advantages and disadvantages of the use of different ionizing radiation processes [42, 44].

Ionizing radiation	Advantages	Disadvantages
γ-Rays	• High penetration and dose uniformity allowing treatment of products of variable sizes, shapes, and densities. Allows food treatment inside packages. • Readily available and low environmental risks.	• 12% of the source must be replaced annually because of its short half-life and a rather slow processing rate.
X-rays	• Relatively high penetrating power (good for thicker foods).	• Poor conversion of accelerated electrons to X-rays (4–6%). • Energy inefficient process.
Linear accelerated electrons	• It can simply be turned off when not in use, does not need to be replenished, has an established history of use, and has a high throughput rate.	• Complexity of the machine and the consequent need for regular maintenance and the large requirements for power and cooling. • Low penetration power, most foods are treated from both sides.

DNA and RNA synthesis, denaturation of enzymes and cell membrane may also be affected. The magnitude of the damage and biological adverse effects are dose-related. Higher irradiation doses result in greater damage of nucleic acids and other biological molecules, effectively killing microorganisms and leading to the products indefinite stability (viruses being excepted). No microbial spoilage or toxicity should become detectable in foods treated with ionizing radiation (radappertization). Lower irradiation doses cause only a substantial decrease in the numbers of viable specific spoilage microorganisms and are sufficient to enhance food quality, resulting in products that last longer under refrigerated conditions (radurization). The absorbed dose is considered to be the most important factor that influences treatment effectiveness, but it also depends on the sensitivity, size and physiologic state of the microorganism, extrinsic characteristic of the environment (pH, temperature, water activity, oxygen content) and intrinsic characteristics of the food (fat content, salt, additives). Different microorganisms have different levels of resistance to radiation, mainly due to their capacity to repair damaged DNA. However increasing doses of radiation can overwhelm these repair mechanisms. The most resistant microorganisms are viruses, followed by bacterial spores and vegetative cells [48–50].

Poultry meat and carcasses, red meats, fishery products and spices and other food ingredients are some examples of food products that can be processed with ionizing radiation, yet the required radiation dose depends on the product. For example, threshold doses for an organoleptically detectable off-flavour is 1.5 kGy in turkey, 1.75 kGy in pork, 2.5 kGy in beef, chicken and shrimp, 4.0 kGy in frog, 6.25 kGy in lamb and 6.5 kGy in horse, when irradiation is applied at temperatures from 5 to 10°C [51].

4.4.5.2 Non-Ionizing Radiation

Ultraviolet radiation (UV) covers a wide range of wavelengths in the non-ionizing region of the electromagnetic spectrum, with wavelengths varying from 100 to 400 nm. Ultraviolet radiation is categorized as UV-A (315 to 400 nm), UV-B (280 to 315 nm) and UV-C (200 to 280 nm). UV-C is known to be germicidal, causing cellular mutation and death, with effects on bacteria, viruses, protozoa, yeasts, moulds and algae [23, 52, 53].

The germicidal effect of radiation is mainly justified by the photochemical reactions that are induced inside the microorganisms.

Lesions in microorganisms are related to DNA absorption of the UV light (254 nm is the most lethal), taking place by cross-linking pyrimidine nucleoside bases (thymine and cytosine) in the same DNA strand. The formation of thymine dimmers in DNA and RNA compromises the cellular functions, and since microorganisms are restricted to realizing the normal transcription and replication of the nucleic acids, eventually, cell death may occur [54–59].

As UV radiation has poor penetration properties, its use is limited to food surfaces. Moreover, the pro-oxidant properties associated with this type of radiation may result in accelerated oxidative rancidity and premature discolouration of fresh red meats that are UV-treated. This method is adequate for the disinfection of surfaces, liquid food, water and air [23].

Microwave and radiofrequency are high frequency energy radiations that belong to the non-ionizing category. These radiations have been used for cooking, drying, blanching, tempering, pasteurizing and thawing. The authorized radiofrequencies are limited to 13.56, 27.12 and 40.68 MHz, in order to prevent interference with communication systems, while microwave frequencies approved for heating are limited to 915 and 2450 MHz for industrial and home use, respectively [48, 60].

Foods are heated by transmitting electromagnetic energy through the product placed between two electrodes, where an electromagnetic field is created by conversion of electric energy. The heating occurs by the movement of positive ions to the negative region and negative ions to the positive region, when an electromagnetic field is applied at radiofrequency wavelengths. This mechanism is also valid in the microwave heating, where charged molecules exposed to fluctuating microwaves oscillate quickly to align dipole molecules according to the polarity of the electromagnetic field; i.e., water and other polar molecules tend to align themselves with the electric field. The rapidly oscillating movement of molecules at such frequencies, due to microwave energy created through intermolecular friction, quickly causes food heating and irreversible inactivation of microorganisms. These thermal treatments usually induce microbial protein and acid nucleic desaturations [14, 23, 61].

The success of radiofrequency heating depends on the dielectric properties of the foods, which are influenced by frequency, temperature, moisture content and composition. Longer wavelengths related to the use of microwaves provide higher penetration depth,

allowing the heating of thicker products, like chicken breast meat [60, 61].

Microwave heating has diverse industrial applications such as: baking, concentrating, cooking, curing, drying, enzyme inactivation (blanching), finish-drying, freeze-drying, heating, pasteurizing, precooking, puffing and foaming, solvent removal, sterilizing, tempering and thawing. Several of these applications may be combined and the choice of processes depends on the desired quality for products and unit operation costs [14].

4.4.6 Other Technologies

4.4.6.1 High Pressure Processing

In high pressure processing (HPP), food is exposed to ultra-high pressure (100–1000 MPa) from a millisecond pulse to over 20 min. During treatment, the products' temperature can be below 0°C or above 100°C depending on the food products requirements [62]. HPP inactivates/denatures proteins and controls important enzymatic reactions responsible for quality degradation [63]. The covalent bonds of food constituents are less affected than weaker bonds possibly due to the low energy levels developed by pressure. Thus, there are no significant losses of sensory properties, and nutrients and food quality can be better preserved than in thermal methods [64, 65]. In addition, HPP can inactivate the vegetative forms of many microorganisms. Cell death occurs because of multiple damages accumulated in different parts of the cell (e.g., cell permeability modifications and functional disruptions) that lead to irreversible leakage of intracellular compounds [66].

The critical process factors related to the success of HPP treatments are: treatment pressure, come-up time to achieve a given pressure, holding time, decompression time, initial temperature of food, process temperature, temperature distribution in the pressure vessel due to the adiabatic heating, characteristics of the food (such as pH, composition and water activity), packing material and type of microorganisms present in food products.

In this technology, food shape or size do not interfere, because pressure acts instantaneously and uniformly throughout the chamber and product. There is only a slight variation of the temperature with the pressure increase (around 3°C per 100 MPa, depending on food composition) [62].

4.4.6.2 Ultrasounds

Ultrasound technology has been studied for several years and can be classified as being of low and high energy (i.e., high and low frequency, respectively). Ultrasound is known to cause chemical and physical changes in biological structures, because of the rapid formation and destruction of cavitation bubbles. The pressure changes during intracellular cavitations, which is the main cause for the bactericidal effect of the ultrasound technology [52]. The micromechanical shocks rapidly form bubbles (that collapse equally fast), which promote the disruption of cellular structures and functional components, thus causing cellular lysis [62]. The effectiveness of ultrasounds on microorganisms' inactivation depends mainly on the bacteria type, intensity level of the treatment, exposure time and temperature.

Ultrasounds are used in areas such as non-destructive testing, cleaning, welding, sonochemistry, and for quality assurance as in non-invasive monitoring. To sanitize solid foods a liquid medium is required to propagate the sound waves [62].

4.4.6.3 Ohmic Heating

Ohmic heating is a thermal process technology where electric energy is passed directly through a liquid or solid product. The resistance imposed by the food leads to the instant generation of heat within the product. The main mechanism of microbial inactivation seems to be thermal. Some researchers, however, suggest that another process such as a mild electroporation mechanism may occur, leading to mild non-thermal cellular damages. This effect may happen during ohmic heating at low frequency (50–60 Hz), which allows cell walls to build up charges and form pores [48, 67, 68]. The major advantage of ohmic heating is related to the uniform heat generation that results in an uniform temperature distribution, avoiding the usual heat damage associated with the excessive surface heating of conventional thermal treatments. As ohmic heating accompanies the current, the heat distribution throughout the product is also faster than in conventional thermal methods. Shorter processing times are required and higher yields are obtained, resulting in better final sensorial and nutritional qualities. Energetically, this process is very efficient once 90% of the energy can be converted into heat [67, 69].

The efficiency of ohmic heating is dependent on composition and physical properties (thermal conductivity, voltage gradient, specific heat capacity and electrical conductivity) of the food to be heated [48, 69, 70]. Currently, ohmic heating is considered as an alternative heating system for continuous flow mode to cook and sterilize liquid foods and solid–liquid mixtures, i.e., pumpable foods [71]. The uses of ohmic treatments to process solid foods are limited to comminuted materials (pulverized or chopped finely) and have not yet attained commercial application in the meat processing industry [67, 72]. One of the greatest limitations of this technology is related to the electrical nature of muscle compounds. Compounds with poor conductivity such as fat, do not generate heat at the same rate of muscles and consequently a cold spot is created. Moreover, characteristics such as muscle fibre direction, size of meat piece and type of meat affect the electrical conductivity, which also limits the use of ohmic heating in solid meats processing [48, 67].

4.5 Chemical Methods of Preservation

Chemical preservatives are used to prevent or slow down microbial spoilage and chemical reactions, maintaining product quality. Additionally, some chemicals impart desirable palatability properties, such as special flavours and texture of cured meat. The combination of chemical preservation methods with other techniques such as chilling, heating or drying, may be quite beneficial, providing especially good protection for perishable foods [1].

4.5.1 Curing

From a historical perspective, food curing may be defined as the addition of salt to meats and fishes for the purpose of preservation. Actually, this process combines the use of several chemicals that produce the colour and flavour that we associate with cured food and continues to be used extensively and successfully.

The ingredients commonly used to cure meat are salt, sugar, nitrite and nitrate, reductants, spices or seasonings and phosphates. Salt (sodium chloride) is the basic compound mostly used in curing processes. It is an effective inhibitor of microorganisms due to their low salt tolerance and reduces water activity in foods. It is essential

in solubilizing myofibrillar muscle proteins, imparts flavour and influences textural characteristics [1, 73]. Salt is often used in conjunction with various sweeteners to counteract its harshness, and to provide roundness and enrichment of flavour. The most commonly used sweeteners by the meat industry are sucrose, dextrose, and corn syrup. Salt should have a high level of purity, because impurities such as metals (copper, iron, and chromium) accelerate the development of lipid oxidation and concomitant rancidity in cured meats. Nitrite and phosphates may be used to retard this effect. Moreover, nitrite reacts with myoglobin to produce the characteristic colour of cured meat, works as an antibacterial agent and has a profound influence on the flavour of cured meats. Phosphates are also used to speed the development of cured colour and have an important impact on water binding and emulsion stability.

The amount of salt used in brines and dry mixtures can vary considerably. However, when extreme levels of salt are used the final product is too salty and, contrarily, too little salt can lead to inadequate protein extraction. In general, salt should be present in finished products at a level of about 2.5%. Reductants are mainly used to speed up the curing process and to make it more uniform. Spices and seasonings only give a characteristic flavour to final products [1, 73, 74].

The most used curing methods are: *(i) dry salt curing*, which uses salt alone or sometimes combined with nitrite or nitrate; *(ii) dry country style curing*, which generally uses salt, sugar, nitrate, and nitrite and may be combined with brine injection for some products; *(iii) brine soaking* in which meat pieces are placed in curing brine, and the cure is allowed to penetrate the entire portion; and *(iv) curing pickle injection*, which uses internal injection of curing ingredients directly into meat pieces [73].

4.5.2 Smoking

Smoking is almost an integral part of curing but it is discussed separately because this method produces some chemicals with important preservative properties, beyond the preservative effect of heating and dehydration processes.

Wood smoke is a complex mixture that results from multiple wood combustion products (gases, ash, tar, phenols, carbonyls, etc.) that are visible as gases (i.e., carbon dioxide, water vapour, nitrogen) and carry unburned solid particles (i.e., ash, resin, tar) as

they escape the combustible heat source. About 400 organic compounds have been unequivocally identified by chromatographic and spectral analytical methods. The exact composition of the smoke is defined not only by the wood source (dryness and contents of hemicelluloses, cellulose, lignin, and resins) and moisture content but also by the combustion temperature and rate of heating, available oxygen in the zone of oxidation of the volatile products and air flow [1, 75, 76]. Smoke contains a disperse or particulate phase, which is not very important for the process since little of it is deposited on products surfaces, and a gas or vapour phase, which is very important since chemical compounds of it will be deposited and react with surface compounds of food products. The smoking process reduces microbial loads not only because of heat, but also due to the deposit of chemicals that have a bacteriostatic effect on the products' surface. Moreover, a compact layer is formed at the surface, which is a physical barrier to microorganisms' survival. The principal chemical compounds found in smoke and their main functions are presented in Table 4.2.

The smoking process may be applied by traditional methods through natural vapour smoke. However, liquid smoke preparations have been extensively used by industries because these preparations allow a more constant, uniform and repeatable production of smoked food, are a faster process, remove potential carcinogenic compounds, and do not require expensive smoke generators [1, 75].

4.5.3 Other Methods/Compounds

Antioxidants such as ascorbic, citric and erythorbic acids or sodium ascorbate and sodium citrate, may be used as preservatives because they slow down or prevent oxidation reactions, avoiding the development of rancidity, off-flavours and discolouration. Vacuum packaging can be additionally applied to lower oxygen concentration, preventing or minimizing oxidative changes. However, many antioxidants scavenge free radicals and interrupt the chain reaction or propagation. Synthetic oxidants such as butylated hydroxyanisole, butylated hydroxytoluene and tert-butylhydroquinone or natural antioxidants such as rosemary, green tea, grape seed extract, and oregano can be used in muscle foods. Yet, their solubility in water in lipid and in the lipid-water interface should be considered [1, 77].

Table 4.2 Functions of major compounds found in smoke [1, 75, 76].

Compounds	Function	Some examples
Acids	Coagulate or denature proteins at products surface Bacteriostatic action	Acetic, benzoic, formic, glycolic, isobutyric, isovaleric, sorbic, valeric
Phenols	Antioxidant function Smoky flavour Bacteriostatic action	Syringol, guaiacol, pyrocatechol, phenol, and their various alkyl derivatives
Alcohols	Function mainly as carriers for other chemicals	Amyl, benzyalcohol, cyclohexanol, ethyl, isobutyl, methyl, propan-2-on-ol, propyl
Carbonyls	Smoke flavour Brownish cast colour Bacteriostatic action	Acetone, benzaldehyde, cyclyopentanone, diacetyl, hexanal, hydroxyacetaldehyde, pentanone, propanal
Polycyclic hydrocarbons	They are not know to impart a flavour or to have a preservative effect	Benz(a)pyrene (carcinogenic)

Sulfites are weak antioxidants but strong antimicrobials, but were removed from the generally recognized as safe (GRAS) list of the American Food and Drug Administration (FDA), a designation that a chemical or substance added to food is considered safe by experts. **Sorbates** are effective inhibitors of mold and yeast growth and are antimicrobial agents in meat, but they also can be harmful for human health. **Sodium lactate** is a natural constituent of meat that was approved for use in mammalian meat and poultry and has antimicrobiological activity against a broad range of microorganisms. **Acidulants** such as vinegar (acetic acid) may also be used to lower pH, avoiding or minimizing microbial growth and preserving foods [1]. Carcass rinses with **organic acids** such as lactic and acetic acids are often carried out to control microorganism proliferation. The antimicrobial mechanism of organic acids is not

completely understood, although the antimicrobial activity seems to be justified by undissociated molecules. **Chlorine** is well known for its impact on microbial survival and was one of the first chemical treatments implemented in industry. The chlorination process usually consists of adding chlorine or sodium or calcium hypochlorite to washing waters. It has been reported that chlorine also has a continued bacteriostatic effect during storage time [78, 79]. **Hydrogen peroxide** is also a well-studied oxidant agent, directly toxic to pathogens. It has both bacteriostatic and bactericidal activity, because it has the capacity to generate other cytotoxic oxidizing species, such as hydroxyl radicals [80]. **Trisodium phosphate**, due to its high pH, has an antimicrobial effect by disrupting cell membranes and increasing DNA water solubility [78]. **Ozone** has also been used to inactivate a spectrum of microorganisms because of its strong oxidation power. It is used in aqueous solution or by spraying on muscle foods, being sometimes associated to thermal treatments [78, 81].

4.6 Microbiological Contributions to Meat Preservation

The endogenous microflora of muscle foods, or intentionally inoculated microorganisms, may contribute to preservation through competition with undesirable microbes or by production of substances that inhibit their growth.

4.6.1 Competition

The nutrient composition of meat, poultry or seafood, rich in proteins, vitamins, minerals and water, is associated with favorable external environmental factors, and may provide an excellent medium for microbial growth. The competition to survive between populations of microorganisms can be strong and specific and may contribute to food preservation since undesirable microbial growth can be controlled. The combination with other preservation techniques, such as cooling or salting can lead to a better control of food microbial contamination and spoilage. The occurrence of microenvironments in food products affects microorganisms' competition. One example is the case of non-vacuum packaged products where near-surface oxygen is easily available, while in deeper regions

oxygen can hardly penetrate. The initial content of microorganisms is also important, because their expression of virulence is more sensitive to environmental aspects than their survival [1].

4.6.2 Fermentation

Meat fermentation is a popular and ancient method of food preservation. It involves complex microbial ecosystems, which combine bacteria, yeasts and moulds. The fermented products can be defined as foods whose specific properties are mainly derived from the effect of microorganisms that are incorporated in raw materials. These foods are characterized by different physicochemical properties and sensory profiles. Some microorganisms and enzymes in meats are responsible for the production of volatile compounds, such as alcohols, aldehydes, and ketones, and for proteolytic and lipolytic activities. The most important microorganisms responsible for product transformation are lactic acid bacteria (mainly *Lactobacillus* spp.) and coagulase-negative cocci (*Staphylococcus* and *Kocuria* spp.) that seem to be autochthonous in this ecosystem and have the capacity to survive during fermentation. In short, fermentation times lactobacilli is predominant in leading to an acid flavour of the products, while in longer fermentation times besides lactic acid bacteria, coagulase-negative cocci and yeasts are also present, producing higher levels of volatile compounds with low sensory thresholds. Moreover, in some specially fermented sausages, some moulds and yeasts proliferate on the product surface, and are responsible for the final characteristics of the product.

Fermentation processes are often combined with acidification and drying to generate an environment that does not allow the growth of pathogenic microorganisms, guaranteeing a products' safety [5, 82–84].

4.6.3 Bacteriocins

Bacteriocins are proteinaceous toxins produced by living organisms that destroy or inhibit the growth of similar or closely related bacterial strains. For example, colicin is an antibacterial substance that is produced by a strain of *E. coli*; nisin is produced by *Lactococcus lactis* and inhibits the growth of gram-positive microorganisms but has no effect against yeasts or moulds; acid produced by lactic acid bacteria also has an inhibitory effect in various organisms [1, 78].

4.7 Hurdle Combinations of Methods

The widely applied food preservation techniques are related to the use of high or low temperatures, reduction of water activity, acidity, redox potential, chemical preservatives and competitive microorganisms [85]. Preservation methods can be applied in combination, generating hurdle impacts on microbial survival. This approach may be more desirable than the application of the processes individually, since less aggressive treatments are usually required when applied in combination. The main objective of hurdles is to achieve a combination with additive or synergetic effects. More than 60 potential hurdles for food preservation have been described [86]. Some hurdles have a conjoint positive impact on the safety and quality of foods. However, some combinations may have a negative effect on products, depending on the intensity of the processes. For instance, fermented sausages should have a low pH, enough to inhibit pathogenic bacteria, but not to affect taste. Hurdles should be adjusted to an optimal range in terms of safety and product quality [85].

The decontamination of carcasses by washing, spraying and/or rinsing with water and/or chemical solutions at different pressures and temperatures has been extensively studied. Lactic and acetic acid solutions are widely used and are effective when applied at 55°C on warm carcasses [87]. Some combinations of other different chemicals have also been tested. For example, a mixture of 0.6% of acetic acid and 0.046% of formic acid seems to be as effective as 1.2% of acetic acid in decontamination of beef cubes. Sequential decontamination treatments have also been successfully tested. Knife trimming and/or water washings lead to higher microbial reductions when associated with methods such as steam pasteurization or lactic acid plus hot water [87].

4.8 Atmosphere Inside Package

The atmosphere inside the food package can be modified by the replacement of the natural composition of air (78% nitrogen, 21% oxygen, 0.03% carbon dioxide and traces of noble gases) by an alternative atmosphere composed with a different gas or gas mixture. The main purposes are for shelf life extension, enhancement of general appearance and quality, protection of foods from external microbial contamination and reduction of preservatives

addition [88, 89]. There are three major techniques that can be used to change the atmosphere surrounding the products inside packages: *(i)* Modified Atmosphere Packaging (MAP) – the food is packaged and the proportion of each gas component is fixed when the mixture is introduced in the pack, but there is no further control during storage; *(ii)* Controlled Atmosphere Packaging (CAP) – the packages containing food products are flushed and filled with a gas mixture and the composition of gases is continuously monitored, controlled and maintained throughout storage; and *(iii)* Vacuum Packaging (VP) – the food is packaged in a package with low oxygen permeability, air is evacuated and the package sealed. The remaining gaseous atmosphere becomes modified indirectly due to the metabolism of the product and/or microorganisms. CAP is mainly used for bulk storage or transport of products, while the MAP system is used for bulk and retail handling as well [28, 90].

Oxygen, nitrogen and carbon dioxide are the gases used in MAP systems. Oxygen maintains the fresh natural colour of the products (e.g., in red meats) and inhibits the growth of strictly anaerobic microorganisms (e.g., in some types of fish).

Nitrogen is used to displace air in packs (mainly oxygen). It also delays oxidative rancidity and inhibits the growth of aerobic microorganisms. Additionally, it prevents the collapse of packs for high moisture and fat-containing foods.

Carbon dioxide is mainly responsible for the bacteriostatic effect through inhibition of the growth of most bacteria and moulds [88, 90].

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5

Food Ingredients

Dongxiao Sun-Waterhouse

The New Zealand Institute for Plant & Food Research Limited, Food Innovation, Mt Albert Research Centre, Auckland, New Zealand

Abstract

Food ingredients are the backbone of the food industry, offering commercial products competitive advantages in the markets. Food additives are used in small amounts to enhance the quality and safety of food products. The development and evolution of food ingredients and additives are market-oriented and customer-focussed, and subject to regulatory approval.

Considerable opportunities exist in ingredient and additive research and development (R&D) for new product development and commercialisation, due to the demand of today's fast moving food markets. Ingredient manufacturers are developing new, improved or cheaper ingredients and additives that can act as replacements for existing counterparts whilst providing increased nutrition and/or food handling benefits such as extended shelf life. Furthermore, foods possess synergistic health benefits beyond just being a source of individual nutrients. In addition, natural health and handling convenience are becoming increasingly important to consumers. In recent years, the demand for natural ingredients and additives by consumers due to their increased health awareness has driven food processors to increasingly utilize natural compounds originated from nature for food and beverage development. Various innovative and specialized functional food ingredients and additives, and processing methods have been developed, which provide a platform for the development of natural functional or wellbeing foods. This chapter firstly provides background information of food ingredients and additives, then reviews some recent work aimed at the development of natural ingredients and additives for food and beverage applications.

**Corresponding author*: dxsun72@hotmail.com

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5.1 Introduction

Today there is a massive range of food products in supermarkets, including dairy products, cereal products, fats and oil products, bakery products and beverages. Each food contains a number of ingredients and additives obtained from dairy-, plant- or animal-based sources. Manufacturing a finished food requires specific ingredients in proper proportions. Food legislation usually requires food products to display a list of ingredients and specification of food additives.

Changing views and perceptions about the effects of certain dietary compounds can profoundly influence the consumption of foods. Consumers are becoming more aware of the relationships between diet and disease, and taking greater responsibility for self-care. A balanced diet provides adequate nutrients and bioactives such as minerals and antioxidants for optimal health. However, consumer preference for fast foods or easy-to-prepare convenience foods means that modern diets prepared using traditional ingredients and additives often lack key nutrients or health-promoting bioactives. Accordingly, consumer demand for foods with health benefits beyond simple nutrition and sustenance ('functional foods') is increasing [1–3]. Consumers actively seek foodstuffs featured in the 'naturalness' and 'wellbeing' categories [4]. Foods and beverages with added healthy ingredients such as fibre, Omega-3, antioxidants and probiotics have entered dairy and non-dairy food sectors such as oil products, baked products, confectionery products and drinks. As a result, the terms 'active ingredient' and 'artificial ingredients' are commonly used to describe how food constituents meet consumer requirements for 'healthiness' and 'naturalness'. Furthermore, many food industries have focused on developing novel food additives, in order to provide the consumer with a greater choice of foods, including natural products, convenience foods and out-of-season foods. Considerable expansion and innovation potential exist in the food additive category. For example, there are increasing uses of natural food additives with low glycaemic index and high soluble solids content in food systems [5].

A wealth of information about food ingredients and additives is available. There already exist comprehensive books in the field of food ingredients and additives, such as the *Dictionary of Food Ingredients* which covers 1,000 food ingredients and additives, including natural ingredients, FDA-approved artificial ingredients, and compounds used in food processing [6]. This chapter aims to highlight some new trends and changes in the food ingredients and additives area. This chapter will be of interest to health conscious food consumers and food manufacturers who want to keep abreast of fundamental advances in the food ingredient and additive sector.

5.2 Useful Terminology and Definitions

In the widest possible sense, an ingredient is a substance that forms part of a mixture. The majority of direct food ingredients are used after they are 'generally recognized as safe' (GRAS) or prior sanctioned. The US Food and Drug Administration (FDA) maintains a list of over 3000 ingredients in its data base *Everything Added to Food in the United States*, many of which we use at home every day (e.g., sugar, baking soda, salt, vanilla, yeast, spices and colours). Ingredient declaration is required on all foods that contain more than one ingredient. Ingredients are listed according to their relative weight in the product as required by the Code of Federal Regulations Title 21 (21 CFR 101.4a). If an ingredient itself consists of more than one ingredient, this ingredient is listed in its percentage of the total product with its constituent ingredients displayed next to its name in brackets. FDA-certified colour additives (by name), sources of protein hydrolysates, and declaration of caseinate as a milk derivative must be included in the ingredient list. Beverages that claim to contain juice must declare the total percentage of juice on the information panel, as required by the Nutrition Labeling and Education Act of 1990 (NLEA). Commercial products that are new to market normally contain at least one novel ingredient to make the products better than existing products.

An active ingredient is that part of a food's formulation that imparts a specific beneficial effect for humans. An example might be a functional ingredient that offers a validated biological property to consumers, such as antioxidant activity. Physiologically active components with positive health outcomes in foods may come from plant, animal or microbial sources. Fortified foods

and naturally healthy products containing bioactives are the two major categories. Thus, food manufacturers have made attempts to add the active ingredients into popular consumer foods. The FDA authorizes qualified health claims for a number of ingredients when used at specific levels, e.g., omega-3 fatty acids, dietary fibre, plant sterols and soy proteins. As opposed to 'natural ingredients', an artificial ingredient usually refers to an ingredient that is synthetic or man-made, such as artificial flavour, colouring, preservative or sugar substitute.

A food additive is defined in Section 201(s) of the Federal Food, Drug and Cosmetic Act (FD&C Act) as any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristic of any food (including any substance intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food; and including any source of radiation intended for any such use); if such substance is not GRAS, sanctioned prior to 1958 or otherwise excluded from the definition of food additives (FDA [7]). This definition excludes ingredients whose use is generally recognized as safe (where government approval is not needed), those ingredients approved for use by FDA or the U.S. Department of Agriculture (USDA) prior to the food additives provisions of law, and colour additives and pesticides where other legal premarket approval requirements apply. **Direct** food additives are those that are added to a food for a specific purpose, and are typically listed on the ingredient label of foods. **Indirect** food additives are those that become part of the food in trace amounts due to its packaging, storage or other handling. Food packaging manufacturers must prove to FDA that all materials coming in contact with food are safe before they are permitted to be used in such a manner (FDA [8]).

New ingredients and additives, such as those called 'functional food ingredients' and 'specialty ingredients', are continuously being developed to meet the requirements of consumers and/or food manufacturers. However, attention should be paid to the limitations/drawbacks and regulatory issues of these new ingredients. A new dietary ingredient is generally deemed adulterated under Section 402(f) by the FD&C Act, unless it meets one of the following requirements: 1) The dietary supplement contains only dietary ingredients which have been present in the food supply as an article used for food in a form in which the food has not been chemically

altered; 2) There is a history of use or other evidence of safety establishing that the dietary ingredient when used under the conditions recommended or suggested in the labelling of the dietary supplement will reasonably be expected to be safe and at least 75 days before being introduced or delivered for introduction into interstate commerce, the manufacturer or distributor of the dietary ingredient or dietary supplement provides the Secretary with information, including any citation to published articles, which is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such dietary ingredient will reasonably be expected to be safe.

5.3 Food Additives

Food additives are substances added to food, other than a basic foodstuff, to preserve a food, enhance stability of a food, and/or facilitate food processing. Sometimes, even if manufacturing conditions are satisfactory, there is a necessity to use chemical additives to impart desired physical properties to the end product. There are six main categories of food additives: colourants, flavouring agents, nutritional additives, preservatives, texturing agents and other miscellaneous additives. An international numbering system (INS) has been developed for food additives by the Codex Alimentarius Commission Committee on Food Additives and Contaminants, and the E system by the European Union.

The most commonly used additives to improve food appearance are colourants. Colour is one of the most important sensory characteristics and has an immediate impact on consumers. Synthetic colourants have been used extensively in the past in processed foods, such as brilliant black BN (E 151), patent blue V (E 131), orange yellow S (E 110), cochineal red A (E 124), and tartrazine (E 102). Recent studies highlight the high risk associated with the long-term consumption of synthetic colourants [9]. As a result, there is increased interest in natural colourants. Natural colourants occur abundantly in fruits and vegetables, and include carotenoids, chlorophyll and flavonoids. Novel plant cultivars such as apple and kiwifruit cultivars with various colours of flesh and skin [10, 11] are good sources of natural colourants. Anthocyanins extracted from blackcurrant have been successfully incorporated into bread, imparting natural purple colouration and antioxidant activity [12]. Colourants are

always part of human foods and thus their consumption impacts on human health. A natural product is not necessarily harmless. The extraction of colourants from natural substances may retain some impurities. Thus, both synthetic and natural colourants are regulated internationally and they must be subjected to premarket safety evaluation, with synthetic colourants further requiring purity certification. Only some of the certified food colourants that are permitted for use in the US can be used by the EU. The concepts of an acceptable daily intake (ADI) level and estimated daily intake (EDI) have been established to provide safety indication to users.

Flavouring agents are a major food additive category, and include sweeteners (low- or non-calorie), flavours (natural or synthetic), and flavour enhancers (E 620-640 or INS 620-642). It is of interest to note that simulated flavours only became available after 1800 when the flavour industry was born, and the flavours produced in the late 19th century were mostly natural (up to 90%). In the 1950s artificial flavours dominated the market (up to 90%), and maintained a significant market share until the 1980s–1990s when natural flavours again became popular (70%). Food regulations forbid practices where flavour might be used to give false values, e.g., forbid the addition of a characterizing flavour to compensate for the lack of natural flavour because of improper food processing. Sweeteners, other than sucrose, are increasingly included into foods due to the health issues related to sucrose. Non-nutritive and nutritive sweeteners differ in their energy content and the amount required for providing the same sweetness. Sweeteners, especially those that are low- or non-calorie, have been used for new foods/beverages that contain reduced energy for diabetic and weight-managed populations. Flavours are used to provide or enhance food flavours. Natural flavours that are native components of fruits, vegetables and other natural materials have attracted interest [11]. Natural flavours are extracted using physical processes such as distillation, cold pressing, maceration, infusion and expression. Artificial flavours contain only a few or only one constituent, whilst a natural flavour may have dozens of constituents. Therefore, artificial flavours that are synthesized by the chemical industries cannot reproduce the same flavour profiles as those occurring in nature. Flavour enhancers are substances that enhance the food's original taste or flavour. They do not impart any flavour of their own but magnify or modify the flavours derived from other food constituents, e.g., monosodium glutamate (E 621). Flavour enhancers function on the basis of taste

synergism, and are regulated as listed food ingredients in the US, safe food additives in Europe, and permitted food additives with no limitation in Japan.

Nutritional additives have been increasingly used in foods to boost nutritional profiles of foods and special dietary purposes, due in part to growing consumer awareness of certain food components (fibre, vitamins, antioxidants, etc). In recent years, vitamins, minerals, amino acids, proteinaceous additives, fatty acids and fibre additives have all increased in use. The stability of additives and their miscibility with the intended food matrix are two essential aspects for selecting a nutritional additive. Some of these nutritional additives may also be used as texturizing agents such as the carbohydrate- and protein-based fat replacers. Thus, the nutritional additives are generally not included as a functional class within the INS or E numbering system except for those with functions under other additive categories like texturizing agents, e.g., pectin. Different extraction/manufacturing methods for pectin would lead to products with varied rheological properties and hydration behaviours (properties which define the usefulness of pectin as a food additive) [13].

Preservatives used in foods fall into one of three categories: antimicrobials (E or INS 200-290), antioxidants (E or INS 300-326), and antibrowning agents (e.g., citric acid E 330, sodium sulphite E 221, and vitamin C E 300). There exist natural ingredients, such as the aqueous extracts from green kiwifruits, which intrinsically possess antimicrobial, antioxidative and antibrowning properties and can be directly used to produce naturally healthy foods [14]. Sun-Waterhouse *et al.* [15–17] proposed the use of phenolic antioxidants to replace synthetic antioxidant butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA) to retard the spoilage of lipid-based products.

Texturing agents are additives that can modify, alter and add texture, body and mouthfeel of foods, and are used in large quantities in food manufacturing. Emulsifiers, stabilisers, thickeners, bulking agents, water-holding agents, phosphates and dough conditioners belong to this additive category. Many texturing agents naturally exist in the extracts of plant and animal materials, offering opportunities for producing ingredients with multi-functions. Emulsifiers (primarily E 431 and E 495, or INS 429-496) are surface-active substances containing at least one residue with hydrophillic affinity and one residue with lipophillic affinity. Emulsifiers are often grouped into anionic, cationic, amphoteric and nonionic emulsifiers, based

on the mechanisms by which they assist stabilisation and formation of emulsion as well as modification of fat crystallization. Stabilisers provide the desired texture in foods and prevent phase separation, evaporation and deterioration of volatile compounds. Food industries use natural gums, natural starches and modified starches as stabilisers for foods like ice cream, yoghurt and confectionery products. Sun-Waterhouse *et al.* [14] used aqueous extracts from green kiwifruit to modify the texture and improve the consumer acceptability of gluten-free bread.

Other miscellaneous additives include processing aids like acidulants, anti-foaming agents, catalysts, chelating agents, clarifiers, enzyme preparations, lubricants, neutralizing agents, propellants, releasing agents and surfactants. For example, magnesium carbonate is added to wafery products, proteolytic enzymes are added to clarify beer and tenderize meat, and calf rennet (EC3.4.4.3) is used to clot milk for cheese making. Some of these additives may remain intact in the end product, whilst others are undetectable. In the confectionery sector, waxes are used on the surfaces of manufacturing equipment, as are lubricants like talc. Acidulants, such as acetic, citric and tartaric acids contribute sour flavours and antimicrobial properties to a food. Each acid has detailed 'Regulatory Use in Foods' instructions.

The use of food additives can be advantageous or detrimental to a food's nutritive properties. Food additives are useful to food manufacturers, and to some extent useful to consumers, if they impart subjective pleasure and nutritional value. Despite the benefits attributed to food additives, there are generally insufficient scientific safety assessments of a food additive. One should consider the toxic potential related to their dose and interactions with food matrix components (including co-existing food additives). In general, adverse reactions are rare from the substances approved by the US FDA, if their use follows the instructions. However, special attention needs to be paid to life-threatening substances such as allergens, nitrate and nitrite preservatives, and sulfite additives. It is worth noting that the procedures through which permission is granted for food additives differ from country to country. The FDA regulates food additives and GRAS substances and determines if new additives will be permitted for food use, or if existing additives can be used in new food products or for new functions (FDA [7]). Authorization for a new food additive in the EU has to be projected via a global strategy, although harmonized additive legislation is in place in the EU (i.e., Directives 94/36/EC and 96/83/EC).

5.4 Novel and Natural Plant-Based Ingredients

Enormous advances have been made in the science of food and nutrition from different dietary sources. There is a growing trend towards the use of plant-based ingredients because of the positive consumer perception of fruits and vegetables and technological advancements in plant material processing (18, 19). Fruits and vegetables are perceived to possess 'naturalness' attributes, diverse nutrient composition and validated health benefits (20–23). This section presents examples that demonstrate the use of novel natural ingredients that contain both active ingredients and natural additives in food systems.

Extraction is a process that is growing in importance and can make a significant contribution to the safe and environmentally friendly processing of food. In plant food, valuable compounds are initially enclosed in cells, which have to be damaged for facilitation of intracellular matter recovery. Easily oxidized substances such as antioxidants must be protected from degradation during extraction (e.g., against air, oxygen, elevated temperature, released oxidative enzymes). When a feed containing a solute is contacted with an extraction medium (e.g., water or a solvent) in which the solute is reasonably soluble, then the solute will distribute itself between the feed and the extraction medium until there is equilibrium between the feed and the extraction medium phases. Ideally, the feed and extraction medium will be essentially immiscible, and the presence of the solute will not change this immiscibility. However, in many biological systems, it is difficult to reach this ideal state. During the process of extraction, one or more compounds ('solutes') transfer from the biological feed material into the extraction medium.

Among the active phytochemical ingredients, polyphenols have attracted great interest due to their positive roles in health enhancement and disease prevention [23, 24]. Polyphenols are secondary metabolites synthesized by plants, including simple molecules such as phenolic acids, biphenyls and flavonoids, and polyphenols [25, 26]. Various polyphenolic extracts have been generated in research and commercial settings. Commercial suppliers include Berryfruit New Zealand; Just the Berries, New Zealand; GNT International, The Netherlands; Penglai Marine BioTech, China; Herbstreith & Fox, Germany.

For the same plant material, different extraction processes will generate extracts containing different compounds or different proportions of same compounds [27]. Moreover, the existing form of

the bioactive compound may alter after a specific extraction process. For example, an alkali pre-treatment of kiwifruit skin and pulp was found to significantly alter the profile of the polyphenols obtained in an extraction process [27].

Consumption of dietary fibre reduces the risk of health problems related to digestion, cardiovascular disease, prostate cancer and obesity [28–30]. Fibre preparation methods that have potential to be scaled up to industrial scale include simple aqueous and ethanolic isolation methods [31–35]. The functionality of dietary fibres depends on the polysaccharide composition, as well as the location and orientation of polysaccharides in the cell-wall networks [31]. The structure and composition of dietary fibre preparations depend largely on their origin and also on the extraction method used [31]. In general, an 'ideal' dietary fibre ingredient should possess the following characteristics: 1) can be commercially sourced at a low price and produced on an industrial scale; 2) contains no nutritionally objectionable components, but a concentrated fibre composition and balanced ratio of soluble and insoluble fibres; 3) is compatible with food processing, and imparts desirable processing functionality and sensory attributes; 4) does not impact adversely on the shelf life of final food products in which it is incorporated, in addition to having a good shelf life itself; and 5) has overall consumer acceptance in terms of origin/sources, wholesomeness and sensory attributes. For example, apple fibre prepared using a scalable and cost-effective aqueous method largely retained the native 3D cell wall network of apple parenchyma cells (Figure 5.1), and contained health-beneficial components such as pectic polysaccharides and bound polyphenols [35].

Regulatory legislation advocating a reduction in the use of organic solvents that are harmful to the environment has led to the

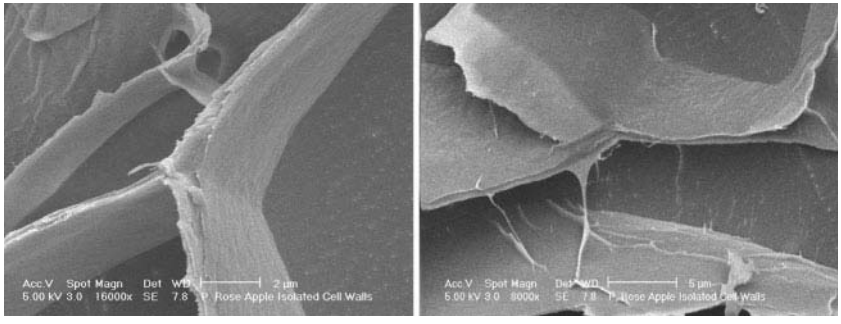

Figure 5.1 Cryo-Scanning Electron Microscope images of isolated apple cell walls prepared using an aqueous method [36].

development of alternative methods for producing pure ingredients without adverse environmental impact [37]. Water is a generally non-selective solvent, though in some cases can be sufficiently selective given the right feed and solute. To meet the growing demand for product purity and energy-efficient processes, with the added advantage of using less organic solvents, alternative processes to traditional solvent extraction methods are being pursued, including aqueous extraction [27], supercritical fluid extraction [38], pulsed electric field [39], solvent-free microwave extraction [40], and high pressure-assisted extraction [41]. Sun-Waterhouse *et al.* [10, 33–35, 42] used aqueous methods to generate natural dietary fibres from apples, feijoa, blackcurrant, boysenberry and onion. These fibre preparations have been found to possess advantages in stabilizing ascorbic acid (Figure 5.2, [33, 34]), exerting gut health functionality [43, 44], and preserving macronutrients like proteins during food processing (Table 5.1, [45]).

Modifying an ingredient processing step can lead to changes in ingredient composition and storage stability. Sun-Waterhouse *et al.* [14] used aqueous extracts from green kiwifruit for preparing gluten-free bread. It was found that the centrifugation speed affected the levels of different compounds in the aqueous extracts (Table 5.2), and the aqueous extracts obtained by centrifugation at 10,000 x*g* and 15,000x*g* were more stable than those obtained at 5,000x*g* and 8,000x*g* during storages at 20°C. This study also showed that it was feasible to generate a natural and bioactive ingredient extract containing polyphenols (also as antimicrobial agents), soluble fibres (also as

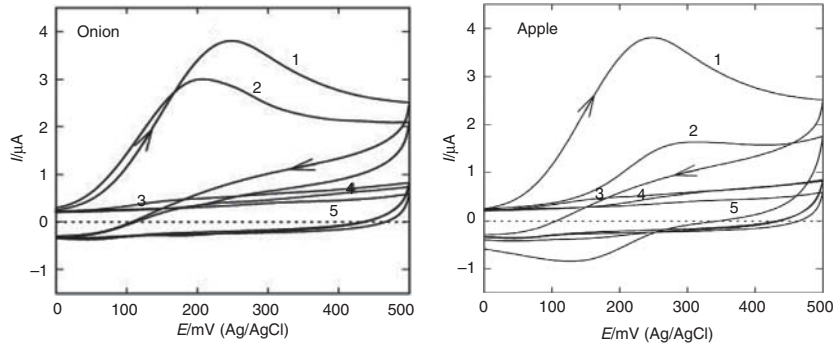

Figure 5.2 Cyclic voltammograms of ascorbic acid in the presence of onion or apple fibre prepared using an aqueous method. Curve: 1, Ascorbic acid before incubation (pH 6.5, 37°C); 2, Antioxidant plus onion or apple fibres after incubation; 3, Ascorbic acid after incubation; 4, onion or apple fibres after incubation; 5, Buffer solution background (HEPES 15 mM).

Table 5.1 Protein, dietary fibre, pectin and phenolic contents of snack bars, with or without added apple fibres.

Snack bar	Protein content (%)	Total dietary fibre content (%)	Pectin soluble fibre (%, as GalA)	Total phenolic content (mg CtE/g bar)
Without added apple fibres	1.07 ± 0.06^{B}	2.47 ± 0.15^{B}	2.30 ± 0.20^{B}	0.50 ± 0.02^{B}
With added apple fibres	2.44 ± 0.03^{A}	5.29 ± 0.35^{A}	2.88 ± 0.10^{A}	0.60 ± 0.03^{A}

Data are expressed as "mean ± standard deviation". CtE and GalA refer to catechin equivalent and galacturonic acid. Different uppercase superscript letters in each column indicate significant differences at $P < 0.05$.

Table 5.2 Total extractable polyphenol, vitamin C and pectic polysaccharide contents in green kiwifruit aqueous extracts.

	Aqueous extracts obtained at different centrifugal force			
Bioactive content	**5,000 × *g***	**8,000 × *g***	**10,000 × *g***	**15,000 × *g***
Total phenolics (mg CtE/mL sample)	1.53 ± 0.05^{C}	2.38 ± 0.09^{A}	1.89 ± 0.05^{B}	1.42 ± 0.09^{D}
Vitamin C (mg/mL sample)	0.66 ± 0.05^{B}	0.97 ± 0.07^{A}	0.97 ± 0.02^{A}	0.64 ± 0.05^{B}
Pectic polysaccharides (as GalA) (% w/w sample)	0.98 ± 0.12^{A}	0.97 ± 0.08^{A}	0.95 ± 0.15^{A}	0.77 ± 0.02^{B}

Data expressed as "mean ± standard deviation". CtE and GalA refer to catechin equivalent and galacturonic acid. Different uppercase superscript letters in the horizontal rows indicate significant differences at $P < 0.05$.

Table 5.3 Total extractable polyphenol contents in the extracts from kiwifruit waste, with or without alkali treatment.

Extraction medium (%v/v Ethanol)	Proportion of the amount of polyphenols in kiwifruit waste extracts (Data expressed as no NaOH : 0.1 M NaOH : 0.5 M NaOH treatment)	
	Skin	Pulp
96.0	0.0137 : 0.0398 : 0.0245 (= 1.0:2.9:1.8)	0.0125 : 0.0347 : 0.0250 (=1.0:2.8:2.0)
49.5	0.0120 : 0.0447 : 0.0214 (= 1.0:3.7:1.8)	0.0058 : 0.0382 : 0.0252 (= 1.0:6.6:4.4)
0.0	0.0143 : 0.0252 : 0.0220 (= 1.0:1.8:1.5)	0.0042 : 0.0254 : 0.0130 (= 1.0:6.1:3.1)

The values in the table are 'Proportion Values' carrying no unit (sum peak areas of the identified compounds, in the cases of no NaOH treatment, 0.1 M NaOH treatment and 0.5 M NaOH treatment, have been normalized against the peak area of the internal standard phlorizin, variation <10%).

texturing agents), and vitamin C (also as an antioxidant). Chemical treatments, such as alkali treatment prior to extraction, facilitated the release of polyphenols from kiwifruit skin or pulp, and altered the total amounts of detected polyphenols as a function of extraction medium, fruit tissue type and alkali concentration (Table 5.3) [27].

Novel plant cultivars are promising sources for manufacturing natural ingredients that possess both sensory and nutritional advantages. For example, new plant cultivars may contain phytochemicals that offer health-promoting properties and preservative effects, and also impart unique and stable colours. If cultivars with particular desirable attributes can be commercialized, less food additives may be required for adding and enhancing colour, flavour and shelf-life of a food. For example, the whole fruit of pink- and red-fleshed apples can be utilized to generate novel juice, fibre and skin polyphenol extract ingredients that are advantageous in sensory, physicochemical and rheological attributes, and health-promoting bioactive composition [10].

Sun-Waterhouse *et al.* [10] found that pink-fleshed apple and red-fleshed apple did not turn brown when sliced or peeled, and their juices did not turn brown during juicing. Polyphenoloxidase (PPO)-catalysed oxidative browning occurs commonly during processing of

apples [46, 47]. Pink-fleshed apple juice had a visually pleasing and stable pink colour, and red-fleshed apple juice had a stable dark-red colour. These results indicate that there may be no need to add anti-browning and colouring agents for the juices from the pink- and red-fleshed apples. Comparative studies further demonstrated differences in the polyphenol profiles of juices between these two novel apple genotypes and the conventional 'white-fleshed' apple (Figure 5.3): The

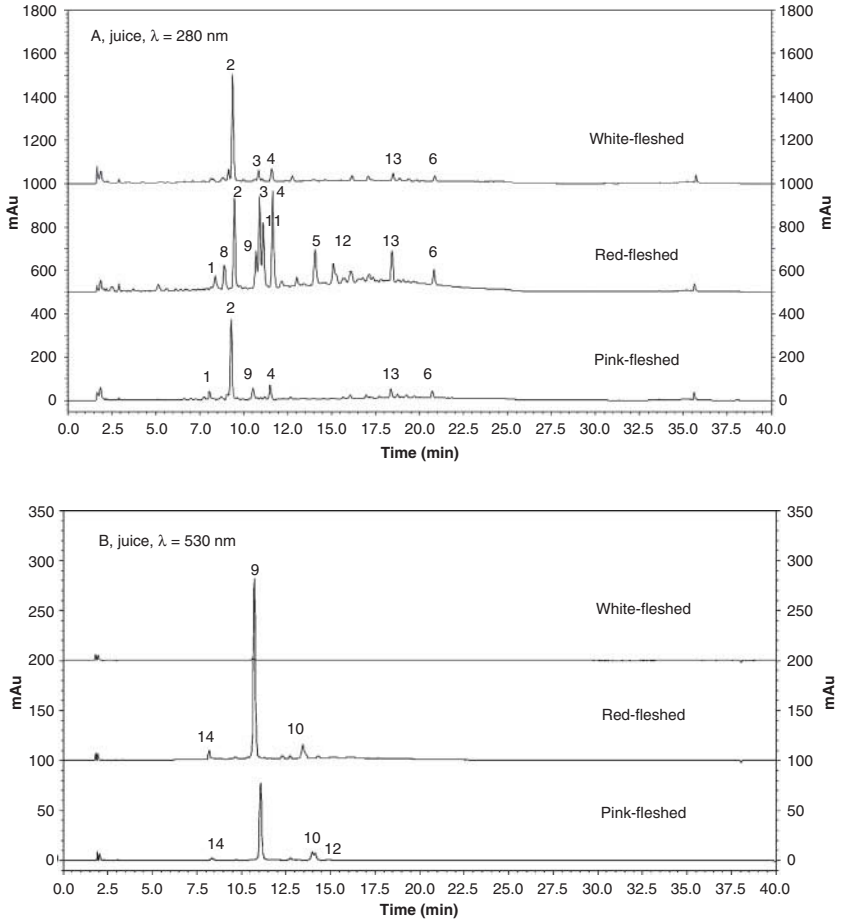

Figure 5.3 HPLC polyphenol profiles of pink-fleshed (PF), red-fleshed (RF) and white-fleshed (WF) apple juices (from bottom to top) at A) λ = 280 nm; B) λ = 530 nm. Peak 1, gallic acid; peak 2, chlorogenic acid; peak 3, caffeic acid; peak 4, epicatechin; peak 5, *p*-coumaric acid; peak 6, phloridzin; peak 8, catechin; peak 9, cyanidin-3-galactoside; peak 10, unknown anthocyanin 1; peak 11, caffeic acid derivative; peak 12, cyanidin-3-glucoside/rutinoside; peak 13, rutin; peak 14, unknown anthocyanin 2.

red-fleshed apple juice contained significantly greater amounts of *p*-coumaric acid, catechin, caffeic acid and its derivative, cyanidin glycosides, phloridzin and rutin; red- and pink-fleshed apple juices contained high concentrations of anthocyanins and vitamin C. These results suggest the minimal need to use additives like stabilisers for the juices from the two novel apple genotypes.

Fibres prepared from the pink- and red-fleshed apple genotypes had higher amounts of bound polypehnol antioxidants and total dietary fibre content compared to a conventional white-fleshed apple. Suspensions containing these fibres also had different viscosity depending on the type of apple genotype and the method used for fibre preparation (Figure 5.4): For the same fibre preparation method, suspensions of the red-fleshed fibres generally had the lowest viscosity; for the same apple genotype, the viscosity of

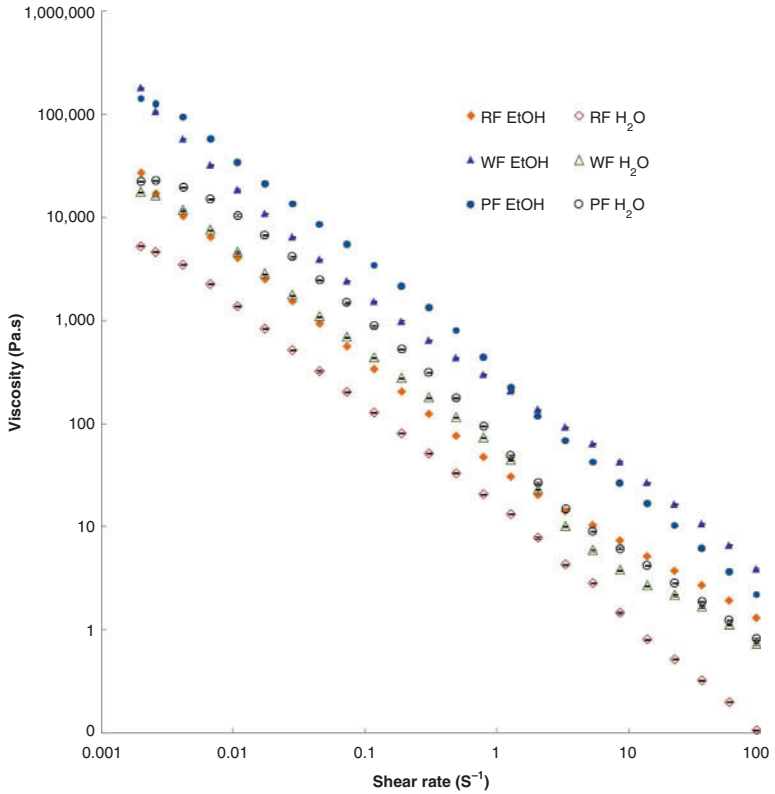

Figure 5.4 Viscosity as a function of shear rate fibres prepared from the pink-fleshed (PF), red-fleshed (RF) and white-fleshed (WF) apple genotypes using the aqueous or ethanolic method, and then reconstituted in water 2%w/w.

the fibre suspensions prepared using the ethanolic method were generally higher than those prepared using the aqueous method. Therefore, fibres prepared from the different apple genotypes, and fibres prepared using different preparation methods, could be selected and used in different food applications (e.g., foods that require different viscosity). The Code of Federal Regulations (Title 21, Part 101.54) allows 'good source of fibre' and 'excellent source of fibre' claims to be made for a product if it is low in fat and provides at least 10% (or 2.5 g), or at least 20% (or 5 g) of the daily value for fibre, respectively. The red-fleshed apple fibre has a greater potential for incorporation at high dosage into foods whilst delivering higher quantities of polyphenol antioxidants.

5.5 Properties and Applications of Plant-Derived Ingredients

The development of minimally-processed fruit and vegetable ingredients facilitates the commercialisation of natural and healthy consumer foods. The incorporation of active or natural ingredients into foods presents technical challenges related to food sensory issues, and bioactive stability and delivery efficiency. These active ingredients sometimes possess properties that make them suitable as food additives. This section showcases the multi-function of plant-derived ingredients in various food applications. These examples show that it is possible to produce foods that meet the growing interests of consumers in 'additive-free' or 'preservative-free' foods, which may ultimately motivate food industries to pursue natural substances as replacements for food additives.

Preventing or minimising lipid oxidation of edible oils is a major focus of research. Adding antioxidant(s) into edible oils has long been used as an approach for improving oil stability. Polyphenol antioxidants are gaining increasing attention in this regard, because of their health-promoting properties, especially the ability to intercept free radicals [48–50]. Polyphenols vary in structure and the extent of hydroxylation of the phenolic rings [51], and may suppress lipid oxidation via donating hydrogen atoms to lipid peroxyl radicals to interfere with the initiation or propagation of primary oxidation [52, 53]. The study of Sun-Waterhouse *et al.* [54] suggests that caffeic acid or *p*-coumaric acid are effective preservative additives for avocado oil. **C**affeic acid and *p*-coumaric acid have relatively

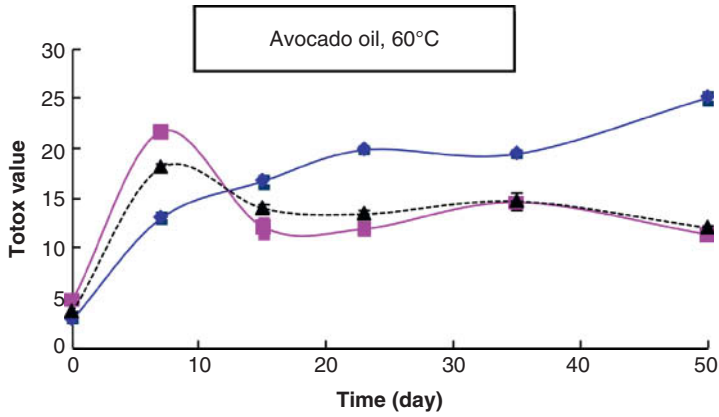

Figure 5.5 Totox values of control and fortified avocado oil stored at 60°C: Control ——●——; Caffeic acid ——■——; *p*-Coumaric acid - - -▲- - -.

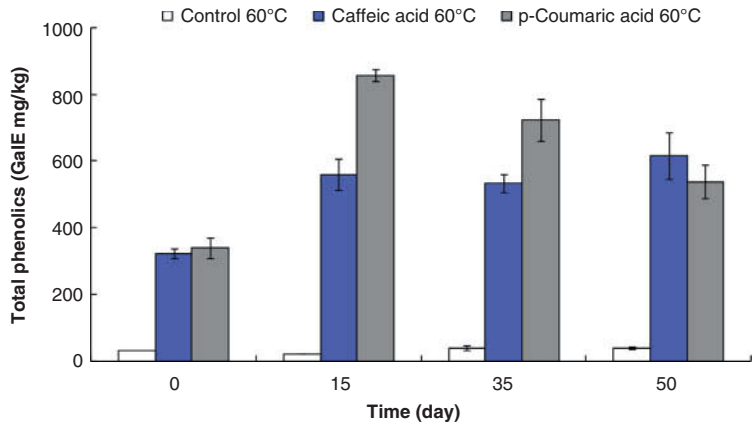

Figure 5.6 Total phenolic content of control and fortified avocado oil stored at 60°C. GalE refers to 'gallic acid equivalent'.

high antioxidant capacity because of their CH=CH-COOH group (25, 55). **C**affeic acid (a dihydroxy derivative) imparts more antioxidant activity than *p*-coumaric acid (a monohydroxy derivative) [56]. The addition of **c**affeic acid or *p*-coumaric acid to avocado oil improved the overall oxidative status of oil, e.g., over a storage period longer than 12 days at 60°C (Figure 5.5), and also increased the total extractable polyphenol content (Figure 5.6). Changes in the Totox values associated with the primary and secondary oxidation stages, were evident by the peroxide values and *p*-anisidine values. Difference in the stability of phenoxy radicals derived from **c**affeic

acid and *p*-coumaric acid would cause different rates of propagation and subsequent oil oxidation reactions [57]. Polyphenol antioxidants would suppress oil deterioration over a specific period of time. This result confirms the feasibility of using polyphenols from natural sources like fruits and vegetables to replace synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and ter-butyl hydroquinone (TBHQ) for improving oil stability. This approach also offers the dual benefit of increasing the nutritional value of the final oil product.

While both polyphenols and dietary fibres are well known individually for their health beneficial effects, these two types of ingredients also contribute to food sensory attributes, sometimes adversely. The co-addition of polyphenols and fibre ingredients into popular consumer foods like pasta represents a convenient way to deliver bioactive ingredients such as polyphenol antioxidants and dietary fibres and utilize them to achieve desired food attributes and quality [58]. Fruit polyphenol extracts, such as anthocyanins in elderberry juice concentrate, imparted dark red-purple colour to pasta, with the colour being different when low or high methoxyl pectin and high maize starch fibre ingredients were also incorporated (Figure 5.7). The fibre ingredients significantly modified the texture of pasta doughs and pasta cooked for 12 minutes in boiling water, with the elderberry juice also playing a role in the firmness of pasta doughs and cooked pasta (Figure 5.8). Interactions between the elderberry constituents and fibre ingredients influenced the total extractable polyphenol contents after pasta preparation and cooking (Figure 5.9).

Ingredient sources, such as fruit or fruit cultivars, play critical roles in the ingredient functionality and derived food properties. In addition, ingredient production method, food formulation and food processing are also important. Sun-Waterhouse *et al.* [59] produced

Figure 5.7 Photographs of dried raw pastas with/without elderberry juice concentrate and pectin. HMS = high maize starch, LM = low methoxyl pectin.

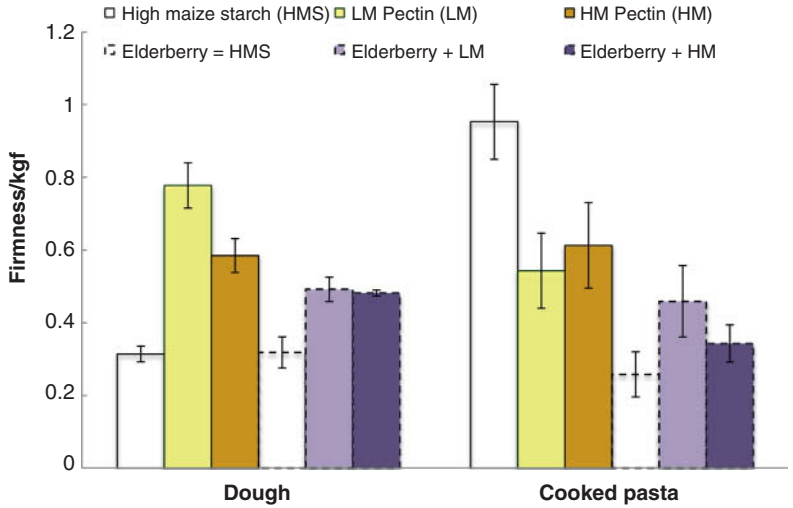

Figure 5.8 Firmness of pasta doughs and cooked pastas.

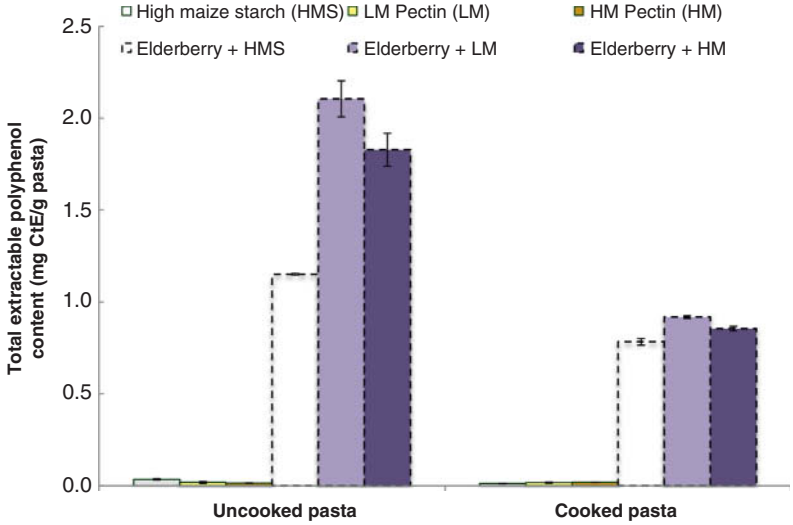

Figure 5.9 Total extractable polyphenol content of uncooked and cooked pastas. CtE refers to catechin equivalent.

aqueous fractions from the purees of green-, gold- or red-fleshed kiwifruits, and then used a substantial amount (up to 49%v/v) of these aqueous juice fractions to produce ice creams in the absence of additional flavouring and colouring additives. The ice creams largely retained the polyphenol and vitamin C contents of the purees, as well as the natural colour and flavour of the kiwifruit

cultivar used. The overrun of the ice cream produced using green, gold and red kiwifruit purees was 90.5, 94.8 and 96.8%, respectively, with the melting rate of green kiwifruit ice cream greater than the other two types of ice cream.

The type of kiwifruit puree influenced the rheological and chemical properties of the ice creams. The relationship between the storage shear modulus G´, which demonstrates the elastic behaviour or the energy storage, and shear strain for the three types of kiwifruit ice cream differed: The G´ of the three ice creams as a function of strain was increased in the order of green > gold > red. A lower G´ of the kiwifruit ice cream might be associated with a stronger fat destabilisation caused by the kiwifruit aqueous juice fraction. The degree of fat destabilisation and the displacement of protein micelles from the fat globule affect the elasticity of ice cream [60–63]. The destabilized fat acts as a cementing agent and provides support to air bubbles primarily lined by proteins, and air bubbles interact with fat in the surrounding medium to manipulate the elasticity component even in low-fat ice creams [63, 64]. Furthermore, the difference in the composition of the kiwifruit aqueous juice fractions resulted in the differences in the total extractable polyphenol contents of the kiwifruit ice creams (Figure 5.10), i.e., in the order of red > gold > green kiwifruit.

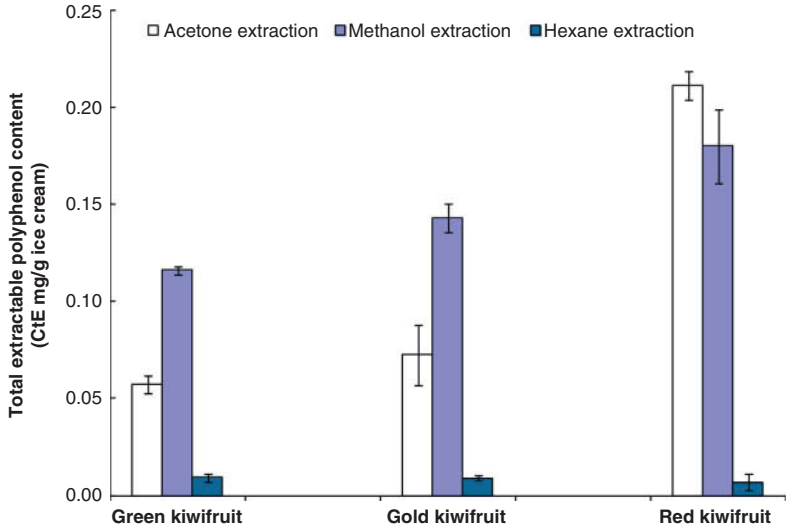

Figure 5.10 Total extractable polyphenol content of ice cream made from kiwifruit with green, gold or red flesh. Error bars are the standard deviation of the mean.

The recovery of the added polyphenols for green, gold and red kiwifruit ice creams was 26, 28 and 43%, respectively. Gold and green kiwifruit contain similar types of cell wall polysaccharides and a high proportion of cellulose, with the gold having a higher proportion of hemicellulosic polysaccharides and lower proportion of pectic polysaccharides [65]. The variations in the composition, acidity and intrinsic enzyme profiles of the three types of kiwifruit aqueous fractions [65–68], led to differences in milk coagulation kinetics (i.e. the gel time and coagulum firming rates) [69], the complexation between kiwifruit polyphenols and ice cream components such as milk proteins and polysaccharides during mixing and processing [70–72], and the physical form of proteins in ice cream [62–64], which ultimately cause a change in ice cream microstructure and the extractability of polyphenols.

5.6 Conclusion and Future Prospects

Over the last few decades, food ingredients and their processing methods have changed significantly, due to increasing consumer awareness about natural foods and the important roles that fruits and vegetables play in human health. A main focus has been increasing production of fruit- and vegetables-based functional foods. Increasingly, natural and/or bioactive ingredients, such as plant-based food ingredients, are being used as food ingredients.

This chapter started with a brief overview of traditional food ingredients and additives as well as their classification, and then explored some new trends and changes in the food ingredients and additives area. A number of examples were provided that demonstrate the desirable attributes and food application potential of novel plant-based ingredients. The chemical, physical, sensory and biological properties of the plant-based ingredients influence the properties of the finished foods. The biological properties of the derived finished foods can be tailored by careful selection of material sources (including plant cultivars and tissue types) and ingredient processing methods. Consideration of the synergies between ingredients/additives and other food or beverage matrix components is key to successful product development. Enhanced food digestibility and nutrient bioavailability, through approaches like optimal extraction and minimal cooking, is the ultimate goal of both ingredient and food development.

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6

Fruits and Fruit Processing

Ariel R. Fontana and Romina P. Monasterio*

Instituto de Biología Agrícola de Mendoza (IBAM) CCT-CONICET Mendoza, Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza, Argentina

Abstract

Fruits and vegetables include a miscellaneous group of plant foods that vary significantly in content of energy and nutrients. Fruits are essential components of a balanced human diet, representing a good source of macro- and micronutrients such as sugars, vitamins, minerals, organic acids, water soluble pigments in dietary fiber, and phytochemicals. There are many nutritional similarities between fruits and vegetables, but there is one important difference with respect to conservation, most fruits are more acidic than the majority of vegetables.

In this chapter, a discussion about fruit and fruit processing strategies for extending shelf life is presented. In this sense, traditional and modern techniques are shown, including low temperature, modified and controlled atmosphere storage, modified atmosphere packaging, edible coatings, heat treatment, drying and modern preservation methods with minimal processing.

Keywords: Controlled atmosphere storage, edible coating, heat treatment, drying, irradiation

6.1 Introduction

A miscellaneous group of plant foods that vary significantly in content of energy and nutrients are included in fruits and vegetables. Fruits are essential components of a balanced human diet,

**Corresponding author*: rmonasterio@mendoza-conicet.gob.ar

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representing a good source of macro- and micronutrients such as sugars, vitamins, minerals, organic acids, water soluble pigments in dietary fiber, and phytochemicals [1]. Low intake of fruits and vegetables is among the top 10 risk factors contributing to mortality, according to evidence presented in the World Health Report 2003. Fruits and vegetables, as part of the daily diet, could help to prevent major non-communicable diseases. Moreover, eating a variety of vegetables and fruits clearly ensures an adequate intake of most micronutrients, dietary fibers and a host of essential non-nutrient substances. The dietary fiber, and fiber intake associated with fruit consumption is linked to lower incidence of cardiovascular disease and obesity. Fruits also provide the diet with important phytochemicals such as polyphenols which are secondary plant metabolites with potential beneficial health effects such as antioxidant activity and antimicrobial, antiviral and anti-inflammatory properties. A WHO/FAO expert consultation report on diet, nutrition and prevention of chronic diseases sets population nutrient goals and recommends the minimum intake of 400 g of fruits and vegetables per day for the prevention of chronic pathologies such as heart diseases, cancer, diabetes and obesity.

Fruits, together with vegetables, are fundamental sources of water-soluble vitamins (vitamin C and group B vitamins), provitamin A, phytosterols, dietary fibers, and minerals for the human diet. Scientific evidence has encouraged the consumption of fruits and vegetables to prevent chronic pathologies such as hypertension [2], coronary heart diseases and the risk of stroke [3]. Recently, the population of developed countries has modified its nutritional habits as a consequence of new life styles. In fact, many studies have reported that the new eating habits related to this life style are causing health problems. An example is the relationship established between fast food with obesity and type-2 diabetes [3, 4]. Unfortunately, the daily intake of vegetables and fruits is estimated to be lower than the doses (400 g, excluding potatoes and other starchy tubers) recommended by the World Health Organization (WHO), and Food and Agriculture Organization (FAO). The food industry is concerned with the elaboration of healthier food products without forgetting the importance of taste and flavor, since they are very important characteristics to consumers [5].

In 2010, the total production of fruits in the world was around 609,213,509 metric tons. An approximate distribution according to the earth's surface is Ocean 0.5–1%; Europe 8–12%; Africa 11–13%;

America 22–35% and Asia 41–51% [6]. In accordance with this distribution of production, and due to the season-dependent production of the majority of fruits, it is important to apply fruit conservation strategies to guarantee consumption of these fruits worldwide.

There are many nutritional similarities between fruits and vegetables, but there is one important difference with respect to conservation, which is that most fruits are more acidic than the majority of vegetables. This difference significantly affects the way that these two types of crops are processed because food pathogenic bacteria cannot grow in acidic fruit products. The majority of fruits are consumed fresh or are minimally industrially processed, which include canned, dried, juice, paste, salad, sauce and soup preparations. Minimally processed and, especially, fresh fruits have a short shelf life since they are subjected to rapid microbial spoilage, and, in some cases, to contamination by pathogens. Cooking and pasteurization as well as the addition of chemical preservatives are the main technological options that guarantee safe vegetables and fruits, but these bring about a number of not always desirable changes in their physical characteristics and chemical composition [7–9]. To reduce these drawbacks, some novel technologies like the high-hydrostatic pressure processing, irradiation and pulsed-electric fields [10], as well as new packaging systems and the use of natural antimicrobial preservatives [10, 11] have been reported as alternatives in recent years. The latest techniques have lower detrimental effects on fruits than the conventional strategies (heat, freezing, etc.) and have attained considerable interest in the related fruit industries.

During the last decade, joint efforts by the packaging and food industries have reduced the amount of food packaging materials. Nevertheless, used packaging materials are still very visible to the consumer in the context of disposal. Environmental issues, awareness against the use of additives in foods, and sustainability of agricultural practices are becoming increasingly important to the consumers. In the same way, modern industries are focused in satisfying these requirements by diminishing the environmental impact of packaging constituents. Consequently, consumer demand may trigger the use of bio-based packaging materials as an alternative to materials produced from nonrenewable resources. Furthermore, the bio-packaging must work as food packaging and meet the requirements of the individual food product [12]. Therefore, edible

coatings can be applied as either a complement or an alternative to modified atmosphere packaging to improve the shelf life of fruits.

6.2 Fruits

Browning and other discolorations, softening, surface dehydration, water loss, translucency, off-flavor and off-odor development, as well as microbial spoilage are some of the most frequent causes of quality loss in fresh-cut products. Nowadays, the use of innovative modified atmospheres and edible coatings stand out among other techniques in the ongoing struggle for maintaining freshness and safety of fresh fruits.

In this section different conservation methods, such as low temperature storage, modified and controlled atmosphere, modified atmosphere packaging and edible coating, along with their advantages and disadvantages, are described.

6.2.1 Low Temperature

Several fruits ripen and deteriorate quickly at ambient temperature. Cold storage is a commonly used alternative to slow these processes and decay development. However, low temperature disorders, chilling injury (classified as internal breakdown), limit the storage life of these fruits. Taste, flavor, and nutritional quality of fruits have been reported to be affected by cold storage temperature and duration.

Low temperature storage cannot be used to its full potential in extending the storage life of all fruits. Some of them including mango, papaya, banana, peach, among others, are susceptible to chilling injury when stored at low temperature (for example at < 13°C for mango) [13–15]. Chilling injury is the major limiting factor in long-term storage, transportation and distribution of the fruit. The most common visual symptoms of chilling injury in fruits are dark coloration of the skin, prominence of lenticels, translucency, mealiness, flesh bleeding, uneven ripening, development of off-flavor and poor fruit quality [15].

Chilling injury occurs when fruits are stored at temperatures below a critical threshold. This is associated with changes in chemical composition of the membranes, particularly its fatty acid composition [13, 16]. The temperature affects the degree of membrane

changes from a gel phase to a liquid crystalline phase. As a result of this transition, the cell membranes lose integrity and cell compartmentation is weakened. The chilling injury is also accompanied by lipid degradation due to the activity of lipoxygenase, for which linoleic and linolenic acids serve as common substrates. The degradation of such polyunsaturated fatty acids yields peroxide ions and malondialdehyde, the product of oxidation injury. Discoloration of the skin or flesh may result from chilling injury, and is caused by browning reactions mediated by peroxidases and polyphenol oxidase [17]. The severity of chilling injury depends on the exposure time, cultivar, and maturity stage [13, 14]. Symptoms of chilling injury appear after transferring the chilled fruit to ambient temperatures (25–28°C) during marketing. Typical chilling injury symptoms include the development of sunken spots or pitting on the skin, discoloration, uneven ripening, inferior fruit flavor, and increased susceptibility to postharvest pathogens. During chilling damage, sugars and organic acids are depleted as a result of increased respiration rates. Superoxide dismutase is an enzyme that converts the radical anion superoxide (O_2^-) to H_2O_2 and O_2. The accumulation of H_2O_2 reactive oxygen species can result in the peroxidation of membrane lipids. The catalase enzyme catalyzes the dismutation of H_2O_2 to H_2O and O_2. Peroxidase facilitates the oxidation of unsaturated fatty acids by singlet oxygen and formation of malondialdehyde. The membrane damage caused by chilling injury can induce the activity of the ethylene-forming enzyme that converts 1-aminocyclopropane-1-carboxylic acid into C_2H_4 [13]. The inhibitory effect of modified atmosphere packaging on chilling injury may be related to reduced water loss and the effects of low O_2 and high CO_2 concentrations on C_2H_4 biosynthesis and sensitivity [18]. Methyl jasmonate was reported to reduce chilling injury via a mechanism that involves an increase in abscisic acid and polyamine levels [17].

Finally, the safe storage temperature is a prerequisite for attaining acceptable taste, flavor and nutritional value of particularly bioactive compounds in the ripened fruit [15].

6.2.2 Modified and Controlled Atmosphere Storage

Modified and controlled atmosphere storage offers an attractive alternative to other previously reported methods for extending shelf life and maintaining fruit quality. In these storage modes, gases

are removed, altered, or added to create an atmosphere around the fruit that varies from ambient air, usually resulting in lower O_2 and elevated CO_2 concentrations. Controlled atmosphere storage has an exact control of the atmospheric composition, while modified atmosphere differs according to fruit respiration rate, package permeability, and storage temperature [17].

Storage atmospheres with moderately low O_2 (2–3%) and high CO_2 (> 5%) levels were reported to inhibit ethylene production and ripening, reduce the respiration rate, maintain color and flesh firmness, retard decay, prevent chilling injury, and thereby extend the storage life of fresh produce. The presence or absence of particular volatile compounds in the storage atmosphere may influence the flavor of fruit after storage [19]. Controlled atmosphere storage enlarges the storage life of many fruits, but with extended storage, the flavor in some fruits may be reduced. This could be due to the reduction in availability of flavor and/or aroma precursors from a reduced turnover of cell lipids. However, gas composition (excessively low O_2 or high CO_2) outside the fruit's tolerance range can cause physiological disorders including uneven ripening, increased susceptibility to decay, off-flavors, and loss of product. Fruits produce different volatile compounds, the relative amounts of which change during storage and ripening. Aldehydes are more prevalent in unripened fruit and esters in the ripened fruit [20].

Successful application of modified and/or controlled atmosphere depends on avoiding mechanical damage and implementing good sanitation practices, temperature management, and humidity control previous and during the application of atmosphere control. Modified or controlled atmosphere conditions are most suitable during sea transport, which is less expensive but of longer duration than air transport.

Controlled atmosphere treatments using CO_2, O_2, and/or N_2, together with controlled temperature and humidity, form an important method for postharvest sterilization against insect-infested fruit [21].

Modified atmosphere has been researched extensively for several years (more than 30 years) and processes such as airtight or hermetic storage have been used successfully to maintain grain [22, 23]. These atmospheres also prevent fungal growth and maintain product quality. An important development stimulating further work on modified atmosphere took place in the United State in 1980 and 1981. The Environmental Protection Agency approved

an exemption from tolerance for CO_2, N_2, and products from an 'inert' gas generator when used to control insects in raw (Federal Register 1980) and processed (Federal Register 1981) agricultural products [22]. The development of this technology has come about mostly because of public concern over the adverse effects of pesticide residues in food and the environment. Although this method has become well established for control of storage pests, its commercial use is still limited to a few countries. Investigations that are more recent have attempted to integrate modified atmosphere application into the twenty-first century version of raw product and manufactured food storage and transportation [22].

Storage of food for extended periods is possible without insect infestation at low water activity, which prevents microbial growth. However, qualitative losses that consist of changes in physical appearance, nutritional degradation due to oxidation, the presence of insects or their residues, or contamination by mold or the presence of mycotoxins still occur. Some of these are difficult to detect visually. If the moisture content maintained is sufficiently low, then insects remain the main concern for the preservation of the quality of durable fruits. Modified atmosphere offers an alternative that is safe and environmentally benign compared to the use of conventional residue-producing chemical fumigants for controlling insect pests attacking stored fresh and processed fruits [16].

Classically, in commercial controlled atmosphere rooms, control of CO_2 levels is achieved by using either an activated carbon scrubber, hydrated lime scrubber, or by purging the rooms with nitrogen. In an activated carbon scrubber, the room atmosphere is passed through a bed of fine mesh activated carbon granules that adsorb the CO_2. The granules may become saturated with CO_2 whereupon the bed is regenerated by purging with air, the released CO_2 being vented outside the store. The timing and number of cycles of adsorption and regeneration maintain the required CO_2 level in the store atmosphere. Lime removes CO_2 from the store atmosphere through the reaction of CO_2 with $Ca(OH)_2$. Lime may be included in the controlled atmosphere room, or in a chamber attached to the room through which the store atmosphere is passed. With the advent of membrane N_2 generators, purging rooms with 98–99.9% N_2 have been used to remove CO_2. The method chosen for CO_2 control depends to some extent on the degree of CO_2 control needed, and/or whether the atmosphere is to be established in a land-based cool store, a sea-freight container, or in the hold of a vessel [19].

6.2.3 Modified Atmosphere Packaging

Packaging under suitable atmosphere conditions can effectively control the growth of microorganisms on the surface of fruits increasing shelf life of the product. The proliferation of aerobic microorganisms can be considerably delayed with reduced O_2 levels. The growth of Gram-negative aerobes such as *Pseudomonas* is specially inhibited, more than for Gram-positive, micro-aerophilic species such as Lactobacillus. Generally high CO_2 concentrations are also effective in controlling the growth of most aerobic microorganisms, specifically Gram-negative bacteria and molds, but fail to inhibit the majority of yeasts in fruit surface [24, 25].

Modified atmosphere packaging, a convenient and cheaper alternative to controlled atmosphere, has also been commercially adopted for storage and transportation of several fruits. Modified atmosphere packaging technology is largely used for minimally processed fruits and vegetables including fresh, 'ready-to-use' vegetables. It involves the use of polymeric films to create a modified atmosphere that is high in CO_2 and low in O_2 concentration around the fruit, reducing the respiration rate and inhibiting ethylene production. There is a large range of polymeric films available in the market with greater flexibility in gas permeability. Fruits are stored in sealed polythene film (40 μm thick low density polyethylene) bags at a suitable temperature. The fruits are removed from the bags to achieve normal ripening [15, 26, 27]. The plastic film also acts as a barrier to minimize water loss from the fruit. Before, the lack of precise control of atmospheric composition in modified atmosphere packaging limited the possibility of manipulation of the concentrations of O_2 and CO_2 near the safe threshold concentrations of these gases [16, 28, 29]. Oxygen, CO_2, and N_2, are most often used in modified atmosphere packaging. The recommended percentage of O_2 in a modified atmosphere for fruits and vegetables for both safety and quality is ranged in between 1 and 5%. Although other gases such as nitrous and nitric oxides, sulphur dioxide, ethylene, chlorine, as well as ozone and propylene oxide have also been investigated, they have not been commercially applied due to safety, regulatory, and cost considerations [27].

An appropriate combination of gas composition, package dimensions and permeability adapted to the respiration of the product is critical to reach a sustainable equilibrium of gas concentrations. This equilibrium must ensure that O_2 levels inside

the packages are high enough to avoid the triggering of anaerobic fermentative processes [24, 25]. It is very important to emphasize that the plastic films used for modified atmosphere packaging create an environmental hazard. Indeed, there is a demand for biodegradable and eco-friendly packaging films to replace synthetic packaging films [24, 30].

The three most important gases used in modified atmosphere packaging are CO_2, O_2 and N_2. The choice of gas is dependent upon the food product being packed. Used individually or in combination, these gases are commonly used to balance safe shelf-life extension with optimal organoleptic properties of the fruit.

Carbon dioxide is a colorless gas with a slight pungent odor at very high concentrations. CO_2 dissolves readily in water to produce carbonic acid (H_2CO_3), increasing the acidity of the fruit by reducing its pH. This has significant implications for modified atmosphere packaging of foods. The high solubility of CO_2 can result in pack collapse due to the reduction of headspace volume [27].

Nitrogen is a moderately non-reactive gas with no odor, taste, or color. It has a low solubility in water and other food constituents. N_2 does not support the growth of aerobic microbes and therefore inhibits the growth aerobic spoilage, but does not prevent the growth of anaerobic bacteria. The low solubility of N_2 in foods can be used to prevent pack collapse by including adequate N_2 concentration in the gas mixture to stabilize the volume reduction due to CO_2 going into the solution [27].

6.2.4 Edible Coatings

Edible films and coatings are a sustainable technology which are applied on many food products with the aim of controlling moisture transfer, gas exchange or oxidation processes. Edible coatings can provide an additional protective coating to products and, at the same time, have the similar effect of modified atmosphere storage by modifying internal gas composition [31]. One of the major advantages of using edible films and coatings is that several active ingredients can be incorporated into the polymer matrix and consumed with the food, thus enhancing safety or even nutritional and sensory attributes of the product [24, 25]. Edible coatings offer an attractive alternative to film packaging due to their environmentally-friendly characteristics [32]. However, it is necessary to carefully control the internal gas composition to achieve satisfactory

results when using edible coatings for fresh products. Application of quality criteria is needed for fruits coated with edible films, as well as the monitoring of quality parameters during the storage period. This includes color changes, firmness loss, ethanol fermentation, decay ratio and weight loss of edible film-coated fruit.

Application of edible coating creates a transparent film on the fruit surface, which acts as a barrier to water and gases, imparts gloss and better color, reduces fruit weight loss, extends storage life and minimizes microbial spoilage. It can also create a modified atmosphere around the fruit similar to controlled atmosphere storage, but the changes in concentrations of O_2 and CO_2 are dependent on temperature and humidity [32, 33]. Edible films and coatings may help to reduce the deleterious effects concomitant with minimal processing, not solely retarding food deterioration and enhancing its quality, but also improving its safety because of their natural biocide activity or by incorporating antimicrobial compounds. In fact, the application of edible coatings to deliver active substances is one of the major advances reached so far to increase the shelf life of fresh-cut produce [12, 30]. The development of new technologies to improve the transport properties of edible coatings is a relevant issue for future research. Also, mechanical, sensory and even functional properties can be dramatically affected by the addition of active additives such as antimicrobials, antioxidants, and nutrients. Thus, research on this topic needs to advance on new coating applications with improved functionality and high sensory performance, which is focused on a commercially realistic scale.

6.3 Fruit Processing

Due to the changes occurring in demographics, lifestyles, and eating habits, consumers are demanding convenient but fresh and healthy foods. In this sense, food industries are driving their traditional production strategies to the application of new and slight preservation techniques, which satisfy the increasing market demands by using fewer additives such as preservatives and humectants, maintaining the higher nutritive value, and natural flavor and taste related with fresh sensory attributes. In order to harmonize or merge these demands without compromising the safety of the products, it is necessary to implement newer preservation technologies in the food industry. Traditional preservation

techniques such as heat treatment greatly affect the appearance, sensorial characteristics, and nutritional value of fruits. Thus, industries are focused on the development of new strategies for conservation of minimally-processed fruits. The starting objective of minimally-processed fruits was to reduce the quality loss that is caused by long and high temperature during heat treatment. The minimally-processed fruit techniques have emerged to replace traditional methods of preservation, while retaining nutritional and sensory quality; these techniques have forced scientists to develop new alternatives for heat treatment without affecting the quality of fruit, while maintaining preservation and shelf life of the products.

These products were introduced in the market as a response to a consumer tendency towards fresh-like, high quality fruits and vegetables, as well as an increase in reputation of ready-to-eat products. Consequently, fresh-cut fruit products need new preservation strategies for keeping the safety and quality of commodities long enough to make their distribution feasible and achievable.

One of the disadvantages of minimal processing is that it does not inactivate completely all microorganisms present in the fresh fruit. Thus, the assurance of the microbiological safety during the shelf life of these foods depends largely on appropriate refrigerated storage and distribution (prevents the growth of hazardous microorganisms) and the restriction of "use-by" time period [34].

6.3.1 Factors Affecting Fruit Conservation Method

A healthy fruit surface contains a lot of microbes, which may be included as the normal microflora or others inoculated during the processing of fresh fruit. The microflora could be formed by plant pathogens, opportunistic pathogens or non-plant pathogenic species associated with human infections after consumption of raw-fruits and/or unpasteurized fruit juices. According to the Center for Disease Control and Prevention (CDCP) more than 50% of outbreaks occur with non-identified etiological agents and tiny culprits. For this reason, fresh fruits need the application of different tools for ensuring a microbiologically safe food as well as for producing the highest quality product. Depending on how the processing is carried out, the latter may result in color, texture, flavor and nutritional quality changes. There are several factors affecting microbial growth which can be divided into extrinsic factors: pH, water activity, redox potential, available nutrients, antimicrobial factors;

extrinsic factors: storage temperature, humidity, and implicit factors: general interference, production of inhibitory substances and biofilm formation. Microorganisms may be controlled through the use of heat, cold, dehydration, acid, sugar, salt, smoke, atmospheric composition and radiation; and the selected method depends on the chemical nature of the fruit. For example, mild heat treatments in the range of 82–93°C are frequently used to destroy bacteria in low-acid foods (pH ≥ 4.6), but to ensure spore destruction temperatures of 121°C wet heat for 15 min or longer are required. High-acid foods (pH < 4.6) require less heat, and often a treatment of 93°C for 15 min will ensure commercial sterility [35]. Other main consideration in the selection of the most appropriate method of food preservation is related to the shelf life required for the final product. If the product will be consumed within the next two weeks after processing, fresh-cut or minimal processing may be sufficient, but refrigeration and other means of preventing microbial growth will be required. On the other hand, if the product is to be stored for a year or more, a process warranting commercial sterility and long-term acceptability is desirable, such as canning or freezing.

6.3.2 Traditional Preservation Methods

Heat treatment. Thermal processing is one of the most common forms of processed fruit preservation because it efficiently reduces microbial population, destroys natural enzymes and renders horticultural products more palatable [35]. This method requires the knowledge of practical and theoretical considerations to achieve satisfactory results in terms of preservation of fruits. The thermal processing methods essentially involve two alternatives: 1) heating unsterile foods in their final containers (canning), or 2) heating foods prior to packaging and then wrapping under sterile conditions (aseptic processing).

The principal disadvantage of thermal processes is that they suffer from the limitations of heat transfer, with a gradient of temperature exposure from the outside to the inside of the food, with over processing causing severe damage to the sensory, nutritional, and functional properties [36].

Freezing. Freezing is one of the best methods for long-term storage of fruits which is based on a lowering of water activity (a_w) to a level that prevents microbial activity and reduces the rates of

chemical reactions. The advantages of freezing are the preservation of some organoleptic attributes such as the original color, flavor and nutritive value of most fruits. When fresh fruits are harvested, they continue undergoing chemical, biochemical and physical changes, which can cause deleterious reactions named as senescence, enzymatic and chemical decay as well as microbial growth. The freezing process reduces food temperature until its thermal center reaches −18°C, with the subsequent crystallization of water which represents between 85–90% of the total composition of fruit and fruit products. From a physical point of view, vegetable tissues can be considered as a dilute aqueous solution, being the natural medium where chemical and biochemical cellular reactions take place and microorganisms grow. The reduction of water activity due to crystallization of water during freezing is responsible for the decay in chemical and biochemical reactions as well as the microbial growth. Other facts that increase the fruit preservation is that freezing involves the use of low temperatures which slow the rates of reactions taking place in tissues. The commercially basic freezing methods are freezing in air, freezing by indirect contact with the refrigerant and freezing by direct immersion in a refrigerating medium [35]. Prior to freezing, most vegetables are exposed to a short blanching treatment with either steam or hot water to inactivate enzymes. While the thermal exposure in frozen vegetables and fruits is relatively low, the freezing and thawing process itself results in significant tissue structure damage, depending on the rate and temperature at which each is applied. This degradation of fruit tissues may allow damage of cellular integrity as well as increase contact of enzymes and nutrient substrates. This fact may result in detrimental effects for fruits including nutrient loss and, additionally, deterioration of texture, color and flavor.

Drying. The increase in world population will strengthen the yet existing population–food imbalance. Besides increasing food supply and limiting population growth, the reduction of food losses which occur throughout food production, harvest, postharvest, and marketing seems to be a viable option.

Fruit drying has a long tradition as a conservation method, being a successful way to preserve nutritive properties for an extended time, and avoiding the loss of fresh fruits after their shelf life has expired. Fruit drying comprises the elimination of different amounts of both free and bound water from fruit. The amount and method of

water removal cause changes in fruit structure which depends on the type of bonding, and also determine the character of the reconstituted dried material before a new utilization. The procedure of drying a moist material and decreasing its water activity refers to the evaporation of bound water from inside the solid material into the atmosphere. This process requires energy and can be done with different types of drying energies such as convective (warm air), contact (cooled surface), irradiative (infrared rays) and excitation (microwave) energies [34].

6.3.3 Modern Preservation Methods with Minimal Processing

In recent years, a number of novel processing technologies have received a lot of interest for their ability to generate microbiologically safe products with extended long shelf life and increased quality when compared with conventional thermally-processed foods. Many of these new approaches were initially classified as 'non-thermal', even though heat may still be generated during application of the processes. In general, the temperatures to which foods are exposed in these advanced processes are relatively low and may be below pasteurization temperatures [37]. For optimizing new tools, an item-by-item approach is required to design processing conditions of the fruits, and it is crucial to know the tolerance level of different microorganisms in specific situations. Non-thermal methods allow the processing of foods at lower temperatures than those used during thermal pasteurization, so flavors, essential nutrients, and vitamins suffer minimal or no changes. Along the same line, the minimally-processed fruit techniques are focused on three approaches that are being investigated. The first refers to the optimization of traditional preservation methods to enhance sensorial, nutritional, and microbiological quality of fruits as well as yield and energy efficiency (i.e., radiofrequency heating, cryogenic freezing, vacuum dehydration). The second tactic is the development of soft processes using novel combinations of traditional physical and chemical preservation strategies to obtain products with quality attributes reminiscent of the fresh or native state of a given fruit but with an extended shelf life (i.e., modified/controlled atmosphere packaging, active packaging techniques). Finally, the last approach focuses on the development of innovative techniques to obtain

novel fruit products with fresh-like quality attributes by using a combination of emerging preservation factors (e.g., non-thermal physical agents such as high hydrostatic pressure, pulsed electric fields, ultrasound, pulsed light, and UV light, and natural antimicrobials, among others), or otherwise combining emerging factors with traditional ones, all of them applied at low doses. In fact, the novel alternative physical agents, intensely investigated in the past two decades, can cause inactivation of microorganisms at ambient or sub-lethal temperatures, avoiding the deleterious effects that severe heating has on fruit quality. These approaches to obtain fruit products of higher perceived quality face different limitations and possibilities for the design, optimization, and experimental assessment, as well as for validation of process conditions.

Pulsed electric fields. The pulsed electric fields (PEF) processing system involves the application of pulses of high voltage (typically 20–80 kV/cm) to foods placed between two electrodes. Pulsed electric fields may be applied in the form of exponentially decaying, square wave, bipolar, or oscillatory pulses and at ambient, subambient, or slightly above-ambient temperature for less than 1 s. There is a set of electrodes introduced into the fluid food in the treatment chamber, a pulse generator, a capacitor, and a switch. The pulse generator charges the capacitor. When it is discharged, the resulting high-energy field pulse creates electrical potential difference across the cell membrane of the suspended cells. When the electrical potential exceeds a certain critical value by a large amount, the change in the cell membrane becomes irreversible, leading to cell death because the critical electrical potential for vegetative bacterial cells is approximately 15 kV cm^{-1} [38]. The microbial inactivation achieved by pulsed electric fields has been explained by different theories. The most studied possibilities are electrical breakdown and electroporation [39]. The impulses of electric high-voltage generate a trans-membrane potential through the membrane of a bacterial cell. If the difference between potential of outer and inner membrane increases above a critical value of about 1 V, an induced polarisation increases the permeability of microbial and plant cell membranes, creating reversible, and/or irreversible pores in the primarily lipid membrane structure, and finally breakdown of the membrane with the consequent microbial cells death is achieved [36].

In this sense, using high field-strength above 10 kV cm^{-1} and duration of the pulses between nano- and microseconds are enough to inactivate vegetative microorganisms in liquid media due to irreversible membrane destruction. However, bacterial spores are not inactivated [37].

There are three types of factors affecting the microbial inactivation with pulsed electric fields: process factors (electric field intensity, pulse width, treatment time and temperature and pulse wave shapes), microbial factors (type, concentration and growth stage of microorganism) and medium factors (pH, antimicrobials and ionic compounds, conductivity and medium ionic strength). Thus, a careful optimization of the method is necessary to achieve satisfactory results in terms of microbes' inactivation and fruit quality.

The principal advantage of PEF are that the energy loss due to heating of foods is minimized, reducing the detrimental changes of the sensory and physical properties of foods. However, the research on real fruit products such as fresh fruit juices is still limited and the majority of studies have been performed using small-size, batch mode equipment. In this sense, it is necessary to evaluate the mentioned experimental advantages of working on continuous-flow and pilot-scale systems.

High hydrostatic pressure. High hydrostatic pressure processing is an advanced technology that is being adopted the quickest by the food industry as a potential alternative to pasteurization of food products. High hydrostatic pressure processing uses water as a medium to transmit pressures from 300 to 700 MPa for several minutes to the food sample, resulting in an inactivation of vegetative cells (reduction in microbial numbers) and enzyme activity [40]. This approach leads to an augmentation of product shelf life by delivering a mild pasteurization effect, which works at ambient temperature without damaging the low molecular weight components, while the nutritional and sensory characteristics of high moisture foods is well maintained in flexible packaging [34]. The degree of microbial inactivation by high hydrostatic pressure is not only dependent on the type of microorganisms but also influenced by the physicochemical environment such as water activity and pH. The inactivation of bacterial spores requires the combination of processing techniques, i.e., pressure with elevated temperatures or other hostile agents. As this can be achieved without heating, the

method is ideally suited for producing the fresh-like, minimally-processed products that consumers are actually demanding. The technique may also be useful in retaining the nutritional quality of foods after processing, especially in foods such as smoothies which contain a high quantity of antioxidant rich fruits [41]. With the aim of fruit shelf-life addition, research should be focused on the development of records to predict the extent of inactivation of specific pathogenic microorganisms under specific conditions, ensuring the reliability of high hydrostatic pressure tools as an alternative to traditional preservation processes for contaminants of target foods. Also, further studies are required to optimise the processing parameters with regard to improved product sensory quality.

Irradiation. Irradiation has gained attention as an effective tool for assuring food safety. The use of irradiation delays ripening, inhibits growth and sprouting and disinfects fresh produce. However, textural alterations induced by irradiation are still one of the main limiting factors for its use in fresh produce. Plant tissues suffer softening with increasing doses of irradiation over critical thresholds. This softening has been attributed to the breakdown of cell wall constituents such as pectin, cellulose and hemicellulose, as well as alteration of semipermeable membranes, which result in structural weakening and loss of turgidity in tissues [42]. Advantageous effects of irradiation on shelf life and quality includes maintaining texture of whole apples after long-term storage. The UV irradiation is most effective for germicidal purposes at a wavelength of 254 nm (UV-C), but information on optimal conditions for different types of media including liquid foods is generally lacking. The bactericidal effect has been suggested to result from photochemical reaction, peroxide and free radical formation, and bacteriophage activation affecting only the surface layers of irradiated foods [43]. However, undesirable changes in texture induced by irradiation are still a limiting factor for its use in fresh-cut produce. Dose limits for detectable flavor degradation and browning may vary greatly, as a function of the differences in composition, variety and maturity of the source fruit [44]. Thus, a more detailed investigation is necessary working with individual fruits to achieve satisfactory results, and a compromise situation reached between organoleptic deterioration and fruit preservation.

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7

Antioxidant Activity of Phytochemicals and Their Method of Analysis

Ashish Rawson[1], Ankit Patras[2,*], B. Dave Oomah[3], Rocio Campos-Vega[4] and Mohammad B. Hossain[5]

[1]School of Natural Sciences, National University of Ireland Galway, Galway, Ireland
[2]Department of Food Science, University of Guelph, Guelph, Ontario, Canada
[3]Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, BC, Canada
[4]Public Food Center Program (PROPAC), Research and Graduate Studies in Food Science, School of Chemistry, Querétaro State University, México
[5]Department of Food Biosciences, Teagasc Food Research Centre, Ashtown, Dublin, Ireland

Abstract

Some of the most exciting core scientific research in the last decade has been the discovery of a group of phytochemicals, which have shown protective effects against cell oxidation. These naturally occurring phytochemicals impart bright colour to fruits, vegetables, pulses or legumes and act as antioxidants in the body by scavenging harmful free radicals, which are implicated in most chronic degenerative diseases. This chapter reviews physico-chemical properties and analytical methods for the antioxidant determination. It also provides insights in descripancies of antioxidant activity measurement of fruits, vegetables, cereals and legumes. Advanced analytical methods for phytochemical identification, characterization and quantification are also discussed to an extent.

Keywords: Antioxidant, phytochemical, health, analytical, method

**Corresponding author*: ankit.patras@gmail.com

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7.1 Introduction

Several epidemiological studies have associated high intake of antioxidant-rich plant products with reduced risk of many chronic diseases, such as atherosclerosis and cancer. The protection provided by fruits and vegetables against several diseases has been attributed to various antioxidants present in these species, such as vitamin C, vitamin E, α-tocopherol, β-carotene and polyphenolic compounds [1–4]. Related studies have also shown that many of these antioxidant compounds exhibit anti-inflammatory, anti-atherosclerotic, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities to a greater or lesser extent [5–8].

The compounds listed above may act independently or in combination as anticancer or cardio-protective agents by a variety of mechanisms. The available scientific data indicates a protective role for plant-based foods against various chronic diseases, i.e., pancreatic, bladder and breast cancers (American Institute of Cancer Research, 1997). This is attributed to an optimal mix of phytochemicals such as natural antioxidants, fibres and other biotive compounds present in foods. In contrast, a recent European Food Safety Authority (EFSA) report has issued negative opinions on the actions of antioxidants in human health. The EFSA panel documented that the claimed effects refer to the protection of body cells and molecules (such as DNA, proteins and lipids) from oxidative damage, including UV-induced oxidative damage. The panel considered that the protection of these molecules from oxidative damage may be a beneficial physiological effect (EFSA, 2010). No human studies investigating the effects of the food(s)/food constituent(s) on reliable markers of oxidative damage to body cells or to molecules such as DNA, proteins and lipids have been provided in relation to any of the health claims evaluated in this opinion (EFSA, 2010). Nevertheless, we believe it is too early to make strong judgements about antioxidants and their biological properties.

The concept of antioxidant activity of unprocessed and processed foods is gaining significant momentum and emerging as an important parameter to assess the quality of the product worldwide. With the expansion of the world global market and fierce competition amongst various multinational companies, the parameter of antioxidant activity will soon secure its place in nutritional labelling with accompanying regulatory guidelines. In this context

development of a practical method of determining the antioxidant activity for industrial use will become imperative. This will give a further boost to the exploitation of fruits and vegetables and development of nutraceuticals and beverages. In this respect, it is of paramount importance to develop analytical methods to quantify antioxidants in foods of plant origin.

This chapter aims to provide a detailed and critical review of the bioassays used to measure total antioxidant activity of foods. Some analytical techniqes for identifying, characterizing and quantifying individual phytochemicals are discussed in this chapter.

7.2 Importance of Antioxidants in Human Health (Their Mechanism of Action)

It is well known that oxygen is the key molecule enabling aerobic metabolism in living organisms. However, its high reactivity, also damages or disrupts bio-molecules by producing reactive oxygen species (ROS). For this reason living organisms have developed a large and complex network of antioxidant molecules and enzymes, able to protect cellular components such as nucleic acids, proteins and lipids from oxidative damage [9]. According to a general definition, antioxidants can slow down or prevent the oxidation of other molecules by removing free radical intermediates. ROS production occurs physiologically during aerobic metabolism and the main role of the antioxidant network present within the cell is to buffer their overproduction, by keeping them at a level where their physiological role can be carried out (i.e., redox signaling). An imbalance of the antioxidant system may cause severe cellular damage resulting in oxidative stress condition, which is often involved in the pathogenesis of important diseases, such as cancer and atherosclerosis. This imbalance is also implicated in other pathological conditions (such as malaria and rheumatoid arthritis) and could play a role in neurodegenerative diseases and the ageing processes [10].

Antioxidants can inhibit or retard oxidation in two ways: either by scavenging free radicals, where the compound is described as a *primary antioxidant*, or by a mechanism that does not involve direct free radical scavenging, where the compound is a *secondary antioxidant*. Primary antioxidants include phenolic compounds such as vitamin E (α-tocopherol) [11]. These components are

consumed during the induction period. Secondary antioxidants function by various mechanisms including binding of metal ions, oxygen scavenging, hydroperoxide conversion to non-radical species, absorbing UV radiation or deactivating singlet oxygen. Normally, secondary antioxidants exhibit antioxidant activity only when a second minor component is present. For example, sequestering agents such as citric acid are effective only in the presence of metal ions, and reducing agents such as ascorbic acid are effective in the presence of tocopherols or other primary antioxidants [10, 12].

Antioxidants exert their effects by different mechanisms [13]:

1. Suppressing formation of active species
2. Scavenging active free radicals
3. Sequestering metal ions
4. Repairing and/or clearing damage
5. Inducing biosynthesis of other antioxidants or defense enzymes

The overall effectiveness of the natural antioxidants (NAO) is dependent on the involvement of the phenolic hydrogen in radical reactions, the stability of the NAO radical formed during radical reactions, and chemical substitutions present on the structure. The substitutions on the structure are probably the most significant contribution to the ability of an NAO to participate in the control of radical reactions, and the formation of resonance-stabilized NAO radicals. The electron-donating ability of methyl, ethyl, and tertiary butyl substitutions at *ortho* and *para* positions to the hydroxyl groups greatly enhance the AOA of phenol. In addition, hydroxyl substitutions at these positions will enhance AOA. *Ortho* substituted phenols, e.g., 1, 2- dihydroxybenzene, tend to form intramolecular hydrogen bonds during radical reactions, which enhance the stability of the phenoxy radical. The presence of a meth-oxy (OCH_3) substitution *ortho* to the hydroxy group is unable to undergo hydrogen bonding resulting in a weaker AOA. Similar AOA would be expected for NAOs having structural characteristics similar to synthetic phenols. NAOs would be expected to participate in radical trapping and singlet oxygen quenching mechanisms. Radical trapping mechanisms can occur via

interactions between radical species such as an antioxidant radical and lipid peroxyl radical. Alternatively, lipid peroxy radicals can interact with electron-dense regions of a molecule. For example, the conjugated polyene system of carotenoids has been found to interact with peroxy radicals. Metal chelating is an example of a secondary antioxidant mechanism by which many NAOs can influence the oxidation process. Metal chelators can stabilize the oxide forms of metals, that is, reduce redox potentials, thus preventing metals from promoting oxidation. In addition, the metal chelators form complexes with the metals making them unavailable to promote oxidation [12].

Examples of NAO are carotenoids that constitute a category. The conjugated polyene system contributes to the singlet oxygen-quenching characteristics of carotenoids. The presence of nine or more double bonds in the carotenoid structure greatly enhances the singlet oxygen-quenching activity. In addition, the oxo groups at the 4 (4´) positions in the β-ionone ring improve AOA. The carbonyl present on the ring stabilizes trapped radicals and therefore reduces the tendency of carotenoids to promote radical reactions. The polyene system can also trap radicals, thus providing additional protective activity [14]. Monophenols and phenolic acids on the other hand donate hydrogen and participate in radical scavenging reactions, whereas the antioxidant activity of tocopherols and tocotrienols is due primarily to the phenolic hydrogen at the C6 position. Also, the AOA of phenolic acids is due to the phenolic hydrogens. Flavonoids are a group of compounds characterized by a C6–C3–C6 configuration and can participate in hydrogen donating, radical scavenging, and metal chelating mechanisms [15, 16] Isoflavones are structurally similar to the flavonoids and found most often in the *Leguminoseae* family. Genistein and the 7-β-glucoside, genistin, have the highest AOA of the isoflavones followed by daidzein and daidzin, formononetin and Biochanin A [17]. The C-7 location has little influence on AOA as noted by the similar AOA of the aglycone and glycoside forms of isoflavones. Compared with flavones, anthocyanidins, metabolic products of flavanones, are less active, and this is attributed to the lack of the C-4 carbonyl that, in conjunction with the C-2: C-3 double bond, plays an important role in AOA [16, 18]. Radical scavenging activity of anthocyanidins is also dependent on the *ortho* OH configuration [18, 19].

7.3 Natural Antioxidants

7.3.1 Sources of Natural Antioxidants

A distinct challenge in the assay of antioxidant activity is the presence within biological systems of at least four general sources of antioxidants: (1) enzymes, for example, superoxide dismutase, glutathione peroxidase, and catalase; (2) large molecules (albumin, ceruloplasmin, ferritin, other proteins); (3) small molecules [ascorbic acid, glutathione, uric acid, tocopherol, carotenoids, (poly)phenols]; and (4) some hormones (estrogen, angiotensin, melatonin,) [20]. Since foods are important as an essential source of such antioxidants, components and trace elements, efficient repair systems are needed to reduce the damage when endogenous antioxidants (e.g. SOD, catalase, GSH) do not completely prevent damage by reactive species *in vivo* [21], and humans must also obtain antioxidants from the diet. The mere mention of natural antioxidants brings about an association with spices and herbs, in that product developer utilize spice and herb extracts as replacements for synthetic antioxidants. However, other natural products such as oilseeds, nuts, cereals, legumes, animal and microbial products can serve as sources of natural antioxidants. Some phytochemicals reported to have antioxidant activity have also been reviewed here.

Herbs. A number of spices and herbs contain compounds that can be removed and added to food systems to prevent oxidation [22–24]. Extracts of many members of the Labiatae (*Lamiaceae*) family (oregano, marjoram, savory, sage, rosemary, thyme, and basil), which are antioxidative, have a high total phenol content [25]. They do not necessarily have high free radical scavenging ability but appear to contain components that function by at least two different antioxidative mechanisms [26,27] observed that, while these antioxidant characteristics are not entirely related to the total phenolic contents, they may be strongly dependent on rosmarinic acid, the major phenolic component. Many herbs (chamomile, rosehip, hawthorn, and lemon verbena) can enhance the activity of antioxidative enzymes such as superoxide dismutase and catalase in a dose-dependent manner and can enhance cell viability and provide protective effects against oxidative stress induced by hydrogen peroxide (in lung fibroblasts [28].

Vitamin E's antioxidant function, as a peroxyl radical scavenger that terminates chain reactions, is well-known and well-described [29].

Vitamin E is the major hydrophobic compound that prevents the propagation of free radical reactions in the lipid counterpart of membranes, vacuoles and plasma lipoprotein [30] and protects against photooxidative stress [31]. In contrast to the described antioxidant property of vitamin E, lipid peroxidation of LDL is faster in presence of α-tocopherol *in vitro* or *in vivo* [32, 33]. It was proposed that peroxidation is propagated within lipoprotein particles by the vitamin E radical (α-tocopheroxyl radical) unless reduced by vitamin C [34].

Nuts. *In vitro* assessment of the antioxidant activity of tree nuts has largely been conducted by examining the ability of extracts to increase the resistance of human plasma or low density lipoprotein (LDL) to oxidation. Extracts of walnuts, almond and almond skins, pistachios, and hazelnuts have been found to increase the lag time of LDL oxidation. Walnut extracts have been reported to inhibit lipid peroxidation reactions as well in human plasma. Pistachios also inhibit lipid peroxidation in bovine liver microsomes. According to Ros [35], the available evidence suggests that while PUFA-rich nuts confer a neutral or minimal effect on oxidative status, the effects of MUFA-rich nuts are more moderate. Indeed, Fito *et al.* [36] reported a significant reduction in circulating oxidized LDL levels among asymptomatic adults, age 55–80 y, 3 mo after consuming a Mediterranean diet including 30 g/d whole nuts mixed at 50% walnuts, 25% almonds, and 25% hazelnuts.

Vitamin C, which includes ascorbic acid and its oxidation product – dehydroascorbic acid, has many biological activities in the human body. Block *et al.* [37] found that vitamin C can reduce levels of C-reactive protein (CRP), a marker of inflammation and possibly a predictor of heart disease. More than 85% of vitamin C in human diets is supplied by fruits and vegetables [38, 39]. Biological function of L-ascorbic acid can be defined as an enzyme cofactor, a radical scavenger, and as a donor/acceptor in electron transport at the plasma membrane. Ascorbic acid is able to scavenge the superoxide and hydroxyl radicals, as well as regenerate α-tocopherol [38].

Kale, red paprika, leaf of parsley, spinach, Lamb's lettuce, carrot, and tomato are very rich in carotenoids (over 10 mg/100 g edible portion). Several carotenoids are precursors of vitamin A (i.e. β-carotene, γ-carotene, and β-cryptoxanthin), and due to conjugated double bonds they are both radical scavengers and quenchers of singlet oxygen [40]. Lower serum β-carotene levels have been linked to

higher rates of cancer and cardiovascular diseases, as well as to increased risk of myocardial infarction among smokers [41].

Cereals are considered functional foods and nutraceuticals because they provide dietary fibre, proteins, energy, minerals, and vitamins, and contain phytoestrogens of the lignin family and several phenolic acids with antioxidant properties required for human health. Whole wheat and wheat bran are the key sources of antioxidants and dietary fibre. Free and esterified phenolic acids have the greatest potential in wheat to benefit health. Phenolic acid in whole wheat bran has strong antioxidant activity compared to whole wheat cereal. Rice bran contains both lutein and zeaxanthin which improves eyesight and reduces the incidence of cataracts. Vitamin-K and inositol hexaphosphate play important roles in preventing kidney stones and are potentially valuable sources of natural antioxidants like tocopherols, tocotrienol and oryzanol. These rice bran components prevent oxidative stress as well as lipid oxidation. Oat is a good source of antioxidants, phytic acid and various phenolic compounds. These antioxidants are concentrated in the outer layer of the kernel and also help maintain the stability of processed oat products and can stabilize oils and fats against rancidity [42].

Legumes. Biologically-active compounds of interest found in leguminous seeds come from many chemical classes and include phenolic acids as well as their derivatives: flavanols, flavan-3-ols, anthocyanins/anthocyanidins, condensed tannins/proanthocyanidins, tocopherols, and vitamin C, among others [43]. Beans also contain many volatile components which may possess some antioxidative activity. Lee *et al.* [44] reported that volatile aroma chemicals found in four different types of beans exhibited antioxidative activities. Aqueous acetone extract rich in phenolics compounds from hulls exhibited high antioxidant and strong inhibitory effect on both cyclooxygenases, COX-1 and COX-2. Anti-inflammatory activity of bean hulls was dependent on their phenolic content and antioxidant activity [45].

7.3.2 Uses of Natural Antioxidants

Considerable interest and recent research in the field of antioxidants have led to better understanding of their mechanisms, mode of action and functionality in the application in food and non-food commodities as well as in biological systems and as dietary

supplements. The use of plants or herbs as antioxidants in processed foods is of increasing importance in the food industry as an alternative to synthetic antioxidants [46]. They tend to be water soluble, as they frequently occur combined as glycosides and are usually located in the cell vacuole [47]. Their significance in the human diet and antimicrobial activity has recently been established [48). The antioxidant properties of these compounds are often claimed for the protective effects of plant-based beverages against cardiovascular disease, certain forms of cancer and photosensitivity reactions [49]. Producers try to prolong the shelf life of edible oils by different techniques, including the addition of antioxidants. The presence of natural antioxidants should always be taken into account, when appropriate levels of added antioxidants are considered. All edible oils contain tocopherols in different total amounts and ratios of α-, β-, γ- and δ-tocopherols. Tocopherols are partially removed during the deodorization process, the last step in refining edible oil. They are often restored by adding nature-identical preparations or natural tocopherol concentrates. Instead of tocopherol concentrates, some specialty oils rich in tocopherols may be added or mixed with edible oils, for example, tocopherol may play a key role in preventing the thermal oxidation of pepper oil during frying [50]. Also, antioxidant properties of many herbs and spices have been reported to be effective in retarding the development of oil rancidity. A new study showed that a combination of sonication treatment and an edible coating of CMC-containing plant extracts could be applied to delay the onset of oxidation of roasted peanuts. Minimizing lipid oxidation s in roasted peanuts is extremely valuable to the peanut industry. This eventually may lead to the preparation of shelf-stable peanut products for the purpose of reaching distant markets to be used during extended storage period [51].

In fact, nuts such as walnuts, hazelnuts, almonds and peanuts are best stored in their shells, where they are sufficiently stable for a year. After shelling, dehulling and roasting they may rapidly deteriorate. About 2–3% oil remains on the surface of peanuts during roasting in hot oil. The best way to avoid this is to add antioxidants into frying oils before the operation. Additions of rice bran oil containing natural antioxidants improve the shelf life of nuts roasted in soybean or rapeseed oils [52]**.** Antioxidants may be added to increase the shelf life of breakfast cereals. Rice bran, stabilized by extrusion, has high antioxidant content, and thus is suitable as a component for breakfast cereals with high stability [53]. Aqueous

extracts from other whole grains or brans, tea extracts and fruit extracts may be used with good results [54]. The catalytic effect of iron is eliminated by phytic acid [55]. Different types of natural antioxidants (tocopherols – alone, in two different doses, or combined with ascorbic acid – and a rosemary extract) were employed in the production of breakfast cereal, to select the most effective ones for achieving low levels of volatile lipid oxidation products, related to off-flavours, after long-term storage. Tocopherols conferred better antioxidant activity than rosemary extract, leading to low levels of off-flavour compounds, with a further improvement provided by the synergistic activity of ascorbic acid [56]. Supplementation of cookies with a mixture of *Petroselini fructus*, *Frangulae cortex*, *Mentha piperitae folium*, *Carvi fructus* can retard the lipid oxidation process and elevate antioxidant activity of the final product [57]. The shelf life of fruits and vegetables is limited by factors other than lipid oxidation, for example, antioxidants are added to fruit and mushrooms to prevent oxidation of polyphenols, the cause of enzymatic browning [58].

Polyphenols may have protective effects against age-related degenerative diseases, since considerable evidence indicates that increased oxidative damage is associated with such conditions. The antimicrobial activity of polyphenols is useful against infectious diseases. For example, the alleged anti-HIV activity would be due to inhibition of enzymes, such as reverse transcriptase, proteinase and integrase, and of CD4 receptors. Polyphenol activity against human and avian influenza viruses appears to be mainly due to the inhibition of viral haemagglutinin, while the activity against cytomegalovirus is attributed to inhibition of epidermal growth factor receptors and immediate early protein function. Animal and *in vitro* models have demonstrated other important effects of polyphenols, such as decreased leukocyte immobilization, apoptosis induction, cell proliferation and angiogenesis inhibition, and phytoestrogenic activity [59]. Cavity oxidative stress and inflammation, consequent to cigarette smoking and cigarettes' deleterious compounds nicotine and acrolein, may be reduced in the presence of green tea polyphenols [60].

Plants may be a source of antifungals since they have to synthesize compounds to resist fungal infections present in their environment. Thus, there has been growing interest in the possible use of plant extracts as natural antifungals, which are less damaging to human health and the environment. Hurdle technology

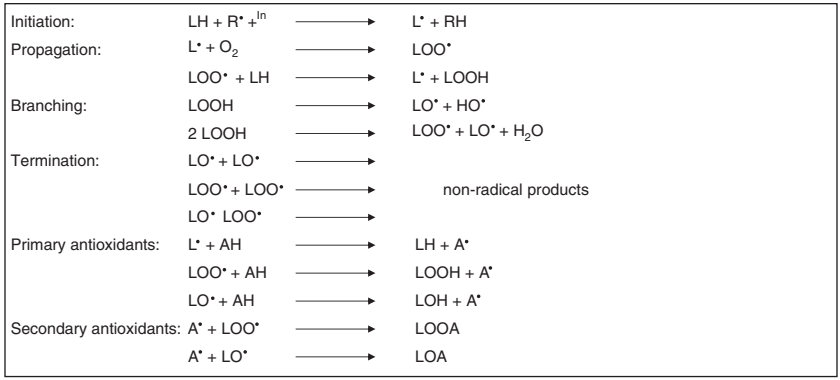
which involves simultaneous multiple preservation approaches has generally met with success in controlling fungal pathogens and maintaining food quality during storage. A combination of preservation treatments allows the required level of protection to be achieved [61].

7.4 Overview of Methods Used to Measure Total Antioxidant Activity

The true definition of antioxidant is often adjusted according to the system. If it is a biological system, a commonly accepted definition of antioxidant is 'any substance that, when present at low concentrations compared to those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate' [62, 63]. According to the definition of Karadag *et al.* [64], the antioxidant prevents the adverse effects of reactive oxygen species (ROS) and reactive nitrogen species (RNS) on normal physiological function in humans. Thus, just the reductants that are able to protect biological system from oxidation can be considered antioxidants [64]. In food systems, antioxidant has been defined as a substance that in small quantities is able to prevent or greatly retard the oxidation of easily oxidisable materials, like fats [65].

Antioxidants can be classified as primary or chain-breaking, and secondary or preventing. The primary antioxidants are active radical scavengers, hydrogen donors or chain reaction breakers, therefore stopping radical chain reactions, delaying or avoiding the initiation step or inhibiting the propagation step (Figure 7.1). Secondary antioxidants are peroxide decomposers, inhibiting the reactive oxidants from being formed (Figure 7.1) [64]. In contrast, pro-oxidant is a toxic substance that causes damage to lipids, proteins and nucleic acids resulting in pathologies [62].

MacDonald-Wicks *et al.* [65] have distinguished the terms antioxidant activity and antioxidant capacity. According to this paper, activity refers to the rate constant of a reaction between a specific antioxidant and a specific oxidant, while capacity is a measure of the amount (in moles) of a given free radical scavenged by a sample. Karadag *et al.* [64] stated that antioxidant capacity is related to 'compounds capable of protecting a biological system from processes or reactions that implicate ROS and RNS'.



LH-substrate molecule; R. – Free Radical; In-Initiator (e.g.light, heat, ionizingradiation;metal ions or metalloproteins); R• -initiating oxidizing radical; L• -allyl radical; LOO• -peroxyl radical; LOOH-hydroperoxides; LO •-alkoxyl radical.

Figure 7.1 Steps of the oxidation mediated by free radicals and action of the antioxidants.

It is very important to emphasise that depending on the conditions of the analytical methods of measurement, different results can be achieved for the same type of food. Moreover, the conditions of the analysis, substrate and antioxidant concentration should simulate the food or biological system [64].

There is a wide range of available methods which differ in regards to the mechanism, oxidant, target, reaction conditions and expression of results. Several reviews have compared these methods [62–69, 70, 71]. For example, Prior *et al.* [69] compared nine methods as to the simplicity of the assay, instrumentation required, biological relevance, mechanism, endpoint, quantitation method and capacity of the assay to measure lipophilic and hydrophilic antioxidants.

Indeed, some authors have tried to select the best method but no consensus was achieved because of limitations, such as determination of hydrophilic antioxidants, difficulty to determine the end point, the light sensitivity of initiators or probes, pH of the analysis, food interferences and the use of different standards to express results. There is no method with all the advantages [64]. The ideal method would be a single, fast and simple assay. Nevertheless, this would not reflect the complexity of antioxidants interactions within food matrices. Therefore, it is recommended to combine assays to study food antioxidant activity [63, 65, 72, 73]. For instance, carotenoids are good quenchers of peroxyl radicals compared to phenolic compounds, but these are exceptional singlet-oxygen scavengers [69, 64].

7.4.1 Measurement of Antioxidant Activity

In the oxidation process, there is the intervention of a substrate, an oxidant, an initiator, intermediates and final products. To determine the antioxidant activity, any of these elements can be measured; consequently there is a wide diversity of methods available.

According to its definition, an antioxidant should have a significant lower concentration than the substrate in the antioxidant activity test. Depending on the type of ROS and target subtrate, a certain antioxidant may play a completely different action or have a completely different role/performance. In line with this, some authors support the use of different methods to measure the antioxidant activity.

Most methods induce oxidation and measure the extent of the consequences/effects. The induction of oxidation is carried out by initiators that include temperature increase, partial pressure of oxygen increase, addition of metal catalysts, like copper and iron, and exposure to light. However, these can negatively influence the results. For example, the oxidation acceleration by light underestimates the effect of chain-breaking antioxidants [63].

The use of substrate is very important in an antioxidant activity test. Depending on the type of substrate and its amount/concentration, different results will be achieved. The application of tests in both aqueous and lypophilic systems has also been described as important in order to study the relative bioactivity of an antioxidant. ABTS method generally does not include a substrate and is considered artificial because it does not represent the real process in food samples.

The measurement of the extent or oxidation rate should also be considered because it can lead to misinterpretation of experimental data. Various approaches can be used like the measurement of the end-point after a fixed time, the reaction rate, and the lag time length, to integrate the end-point versus time curve (this is used when reaction kinetics is not of simple order).

Antolovich *et al*. [63] summarized the methods for expressing results of antioxidant activity. Due to this broad variety, comparison among methods can be difficult.

7.4.2 Assays Involving a Biological Substrate

Assays involving biological substrates have the advantage of being closer to an *in vivo* situation, where both aqueous and a lipid phase

are present and take into account the solubilities and partitioning between different phases. One of these assays measures the inhibition of ascorbate/iron-induced lipid peroxidation of cell or liver microsomes [74,75].

In this procedure described by Lana and Tijskens [75], rat liver microsomes are diluted with Tris-HCl/KCl buffer (50 mM, pH 7.4) and the pellet resuspended with the same buffer and incubated in well-plates after centrifugation. Afterwards, sample extracts, ascorbic acid and $FeSO_4$ are added and the reaction stops with the addition of thiobarbituric acid/trichloroacetic acid (TCA)-HCl (16.8% w/v TCA in 0.125 N HCl). Solutions are centrifuged and the absorption read at 540 nm (colour) *versus* 620 nm (turbidity). This method was applied to tomatoes [75], apple [76] and apple juice [77]. This method was valid for sugar-rich foods but good controls are needed to assure that there is no interference of proteins, lipids, organic acids or coloured compounds with the reaction products. Other assays that employ biological subtrates include the inhibition of human LDL oxidation [78–80] and the lecithin-liposome oxidation assay [78, 81, 82], both catalysed by copper. These models are important because LDL oxidation is related with coronary disease and liposomes oxidation with food oxidation.

7.4.3 Assays Involving a Non-Biological Substrate

7.4.3.1 Electron and Hydrogen Transfer Assays

Assays for measurement of antioxidant activity may involve Hydrogen Atom Transfer (HAT) or Single Electron Transfer (SET). These two mechanisms generally occur simultaneously and the prevalence of one of them depends on the structure of the antioxidant and pH. The mechanism and antioxidants efficiency are mainly determined by two factors: the bond dissociation energy (BDE) and the ionisation potential (IP) [69, 64]. HAT methods measure the capacity of an antioxidant (AH, a hydrogen donor) to quench free radicals by hydrogen donation.

$$X^{\bullet} + AH \rightarrow XH + A^{\bullet}$$

In the HAT-based assays, the reactivity is determined by the BDE of the H donating group of the antioxidant and it is higher for compounds with ΔBDE ~-10 kcal/mol and ΔIP <-36 kcal/mol [69].

HAT assays depend on the solvent, pH and are affected by the presence of reducing agents like metals. HAT reactions are generally quite fast and the quantitation derives from the kinetic curves [64].

HAT assays include the oxygen radical absorbance capacity (ORAC), the total peroxyl radical-trapping antioxidant parameter assay (TRAP) and the crocin-bleaching assay.

SET methods measure the capacity of a potential antioxidant to transfer one electron to reduce a compound:

$$X^{\bullet} + AH \rightarrow X^{-} + AH^{\bullet+}$$

$$H_2O$$

$$AH^{\bullet+} \leftrightarrow A^{\bullet} + H_3O^{+}$$

$$X^{-} + H_3O^{+} \rightarrow XH + H_2O$$

$$M(III) + AH \rightarrow AH^{+} + M(II)$$

In the SET-based assays, the reactivity is determined by the deprotonation and IP of the functional group. These assays are pH-dependent [65]. The higher the pH, the lower IP values are and deprotonation increases. In compounds with ΔIP >-45 kcal/mol, the major reaction mechanism is SET [69].

SET reactions are usually slow and negatively affected by trace components and contaminants, especially metals [69]. Generally, these reactions measure relative percent decrease in product instead of kinetics or total antioxidant capacity [64].

SET assays include the ferric ion reducing antioxidant power (FRAP) and the copper reduction capacity assay. Trolox equivalent antioxidant capacity (TEAC) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assays are usually classified as SET but both mechanisms may be used [69]. HAT and SET are competitive reactions but it has been demonstrated that HAT is dominant in biological redox reactions [64].

7.4.3.1.1 Reduction of the Fremy's Radical

The Fremy's radical assay is an indirect method to determine 'chain-breaking antioxidant activity' in food, and is based on the capability of the Fremy's stable free radical to react with H-donors. The Fremy's radical (potassium nitrosodisulfonate) is a specific oxidizing salt which converts phenols into quinines [83]. The concentration of the Fremy's radical is monitored by electron spin resonance

(ESR) spectroscopy. A low signal indicates the detection of low amounts of radicals and therefore, an antioxidant and dominating prooxidant effect of the extracts [84].

In particular, the method was applied to wine [85] extracts made from cherry liqueur pomace [86], fruit juices [87], coffee [84] and Scotch whiskeys [88].

Rϕdtjer *et al.* [86] have used the Fremy's radical to calculate an extinction coefficient for the anion of the Fremy's salt (ε_{270} = 933 $M^{-1}cm^{-1}$).

Gardner *et al.* [87] pointed out the advantages of this assay: it is very sensitive, allowing detection at a sub-micromolar level; analysis can be carried out on turbid or highly-coloured solution and radicals have well-defined spectra, allowing clear resolution from radical intermediates which may be formed during the oxidation process.

7.4.3.1.2 Copper (II) Reduction Capacity

This method is a variant of FRAP assay, using copper (Cu) instead of iron (Fe). It is based on the reduction of Cu (II) to Cu (I) by the action of the reductants (antioxidants) present in a sample [69, 67]. This method, however, has not been broadly used [65]. Several methods based on the reduction of Cu have been described in the literature using different chromogenic reagents. For instance, Bioxytech AOP-490 assay uses bathocuproine (2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline). This compound forms a complex with Cu (I), in the proportion 2:1, with λ_{max} = 490 nm.

Zaporozhets *et al.* (2004) [89] used tetrabenzo[b,f,j,n] [1, 5, 9–13] tetraazacyclohexadecine. The complex formed with Cu (II) is immobilised on silica gel and has an absorbance maximum at 712 nm [89, 67]. The CUPRAC (Cupric Reducing Antioxidant Capacity) assay uses neocuproine (2,9-dimethyl-1,10-phenanthroline) which forms a complex with Cu(I) with λ_{max} = 450 nm [90].

The CUPRAC assay presents several advantages; the CUPRAC reagent is more stable, economic and accessible than other chromogenic reagents. Unlike FRAP, it is sensitive to thiol-type antioxidants like glutathione. The reaction occurs, near physiological pH (pH 7), while FRAP reaction is generally at acidic pH (pH 3.6) and Folin methods at basic pH (pH 10) [90, 64].

The method is linear over a wide range of concentrations and can measure both hydrophilic and lipophilic antioxidants [91]. Reactions with copper are more selective than with iron because of

the lower redox potential. For instance, sugars and citric acid interfere with FRAP but not with CUPRAC. Another advantage of copper reduction assay is obtaining faster reaction kinetics than iron with little interference [91].

In the CUPRAC assay, the antioxidant solution is mixed with aqueous $CuCl_2$, alcoholic neocuproine and ammonium acetate at pH 7 [64].

The antioxidant solution may or may not have been submitted to acid hydrolysis. For instance, flavonoid glycosides require an acid hydrolysis to obtain their correspondent aglycones [92]. The reaction time depends on the compounds and it is completed in a few minutes for ascorbic acid, uric acid, gallic acid and quercetin. However, some complex molecules require 30–60 min [69]. The highest CUPRAC capacities were obtained in the following order: epicatechin gallate > epigallocatechin gallate > quercetin > fisetin > epigallocatechin > catechin > caffeic acid. This depends on the number and position of –OH groups as the level of conjugation of the molecule (Apak et al., 2008 [90]). The CUPRAC method involves a simple sample preparation and the procedure is flexible and suitable for automation [92].

7.4.3.1.3 Ferrous Oxidation–Xylenol Orange (FOX) Assay

The FOX assay measures the hydroperoxides (ROOHs) which are the initial stable products formed during peroxidation of unsaturated lipids such as fatty acids and cholesterol (Nourooz-Zadeh, 1999 [93]). The assay is based on the oxidation of ferrous (Fe^{2+}) to ferric (Fe^{3+}) ions by ROOHs under acidic conditions. In the presence of xylenol orange (XO) (o-cresolsulfonphthalein-3,3-bis(methylliminodiacetic acid sodium salt)) react with ferric ion to originate blue-purple colour complex with a maximum absorbance at 560 nm.

$$\text{Fe (II)} \xrightarrow[\text{Hydroperoxides}]{} \text{Fe (III)} \xrightarrow[\text{XO}]{} \text{Fe(III)-XO}$$

Approximately three moles of Fe (III) are formed per mole of ROOH [94, 95]. The exact stoichiometry is unclear [96, 97].

Acyl and alkylhydroperoxides and H_2O_2 have the same reactivity as FOX assay, while endoperoxides are almost totally unreactive [95]. There are two versions of FOX assays, FOX 1 and FOX 2. According to Nourooz-Zahed [98], FOX 1 version is suitable for the determination of very low (<μM) levels of hydrogen peroxide in aqueous buffers [99] and FOX 2, for the measurement of lipid

hydroperoxides. Hereafter, FOX 2 will be just named as FOX. In the case of FOX, generally aqueous ferrous ammonium sulphate, methanolic H_2SO_4, methanolic XO, methanol and sample extract in methanol are added. The mixture is incubated at room temperature during the time needed to reach a stable end-point and the absorbance is measured against the blank. Some authors carry out lipid extractions prior to FOX method and others do not [100]. The effect of the acid used in the analytical protocol has been studied. H_2SO_4 [94, 98, 100, 101] has often been used but HCl has also been tested. Higher sensitivity and better precision were observed for H_2SO_4 than for HCl [94, 96] replaced H_2SO_4 by perchloric acid (110 mM) because they claim it increases the range of optimum pH of the assay, allowing a wider pH shift tolerance and increases the molar absorption coefficients of the LOOH.

Different solvents have been used in the FOX assays. Methanol is the most commonly used [94, 98, 100–102]; chloroform/methanol [103]; dichloromethane/methanol (DCM/MeOH) or dichloromethane/ethanol (DCM/EtOH). DCM/EtOH (3:2) is suitable to dissolve large amounts of lipid extract (at least, up to 25 mg lipid extract/ mL reaction medium) and allows the development of the colourimetric reaction with high sensitivity.

The hydroperoxide value of a sample can be expressed as milligrams of cumene hydroperoxide per kilogram of sample [94].

Prior to analysis, samples are treated with enzymes to eliminate H_2O_2, like catalase [98, 100] and hydroperoxides (glutathione, glutathione peroxidise or phospholipase A_2) [101] or triphenylphosphine (TPP) to reduce hydroperoxides to their corresponding alcohols [98, 100] with no effect on H_2O_2.

The aim is to avoid the interference of H_2O_2 or other interferences. Glutathione, cystein, uric acid and ascorbic acid have been described as interferences [94], as well as other compounds that compete with XO for the ferric ions generated by oxidation of ferrous ions mediated by hydroperoxides [94].

FOX is a precise and simple method but the amount of extract and the incubation time have to be adapted for each sample [94]. FOX was also reported as being highly specific [94]. FOX was compared with iodometric method which also measures hydroperoxides that oxidise iodide to iodine [98]. A good agreement between iodometric assay (IA) and FOX methods was cited [100]. However, because iodine reacts with serum protein, the application of IA to human serum is not reliable and this may explain why IA values are

often lower than FOX values [97, 100]. Moreover, the FOX method is sensitive (measures concentrations of 5 μM LOOH), inexpensive, rapid, insensitive to ambient oxygen or light levels and does not require special reaction conditions [100].

Some authors have found unexpected differences among batches of XO from different suppliers [94, 104]. Therefore, it is recommended to use the same batch of XO for all assays.

FOX assay has been applied, for instance, to lipoprotein and liposomes [95, 101], plasma [98, 105], vegetable oils [98], soybean oils [97], plant tissue [100], fried snacks [106] and dark chicken meat [94].

7.4.3.1.4 Ferric Thiocyanate (FTC) Assay

The ferric thiocyanate method determines the amount of peroxide at the initial stage of lipid peroxidation. The peroxide reacts with ferrous chloride ($FeCl_2$) producing a red colour ferric chloride dye. Generally, the sample is dissolved in methanol, and linoleic acid solution in ethanol. Then, phosphate buffer and water are added and the mixture is kept at 40°C for 72 h. An aliquot is taken and diluted with ethanol (75%) followed by addition of ammonium thiocyanate (30%). After 3 min, $FeCl_2$ (0.02 M) in HCl (3.5%) is added and the absorbance measured at 500 nm [107–109] every 24 h until absorbance of the control has reached the maximum.The same procedure is used with solvent instead of sample for the control, and, of vitamin E (4 mg) as standard replaces the sample [110].

Recently, some studies have used this technique to evaluate the antioxidant capacity in different matrices such as citrus by-products [109]; gingers [111]; Malay traditional vegetables [107]; rosemary extract, blackseed essential oil, carnosic acid, rosmarinic acid and sesamol [108]; edible seaweed (Kappaphycus alvarezzi) [112]; sugar cane bagasse [113]; hazelnut skin [114]; wines, grape juices [115]; extracts from *Platycodon grandiflorum* A. De Condolle roots (plants used both as a herbal medicine and food in Asia) [116]), and; sweet potatoes [117].

Results are expressed as the % inhibition of lipid peroxidation (IP %), which is the concentration required to achieve 50% inhibition of linoleic acid oxidation, calculated after plotting the IP % against sample concentration [115].

7.4.3.1.5 Hydroxyl Radical Scavenging Deoxyribose Assay

The deoxyribose assay for detection of hydroxyl radical (•OH) scavenging activity described by Halliwell and coworkers [118] was designed as a relatively simple and cheap spectrophotometric

alternative to pulse radiolysis for the determination of the rate constants of •OH scavenging compounds reacting with hydroxyl radicals. Briefly, the assay relies on the generation of •OH via the Fenton reaction:

$$Fe^{2+} + H_2O_2 \rightarrow \bullet OH + OH^- + Fe^{3+}.$$

Upon reaction of •OH with deoxyribose under neutral pH conditions, the sugar degrades and forms malondialdehyde ($O{=}CHCH_2HC{=}O$, MDA) with heating under acidic conditions. The MDA produces a pink-coloured chromogen when heated in the presence of 2-thiobarbituric acid (TBA) which can then be detected at 532 nm. The rate of deoxyribose degradation in this assay is enhanced by the inclusion of ascorbic acid which reduces ferric to ferrous ions to facilitate the Fenton reaction above.

Typical reaction conditions (final concentrations) consist of: 3.6 mM 2-deoxy-D-ribose, 100 µM ethylene diamine tetra acetic acid (EDTA)-Na_2 dihydrate, 1 mM H_2O_2, 100 µM L-ascorbic acid, 100 µM $FeCl_3{\cdot}6H_2O$ in 25 mM phosphate buffer, pH 7.4 in a 1.0 mL volume. Following incubation at 37°C, 1 h, 1mL 1% (w/v) 2-thiobarbituric acid (TBA) in 0.05 M NaOH and 1mL 10% (w/v) trichloroacetic acid (TCA) are added, followed by heating in a boiling water bath, 15 min. Once cooled, sample absorbances are read at 532 nm. According to Halliwell and coworkers [118], the reaction of •OH with deoxyribose is the rate determining step in the formation of the degradation products resulting in MDA formation. Moreover, the reaction of •OH has been demonstrated to be constant over time. Thus, antioxidant molecules which scavenge •OH will compete with deoxyribose and thereby decrease the final amount of the pink chromogen formed. Interestingly, EDTA itself is an •OH scavenger in this assay [118], therefore, only those •OH which are not scavenged by EDTA go on to degrade the deoxyribose. In order to determine the second order rate constant for the reaction of •OH scavenging antioxidant molecules (S) with hydroxyl radicals in the presence of deoxyribose (DR), the following equation is used [118]:

$$\frac{1}{A} = \frac{1}{A^o}\left(1 + \frac{ks[S]}{k\mathrm{DR}[\mathrm{DR}]}\right) \tag{7.1}$$

where A = absorbance in the presence of S at concentration [S]; A^o = absorbance in the absence of S; k_s = rate of reaction of S with •OH; k_{DR} = rate of reaction of DR with •OH. Thus, a plot of $1/A$ versus [S]

generates a straight line slope which can then be used to calculate the rate constant of the reaction of S with •OH as follows:

$$k_s = \text{slope} \times k_{DR} \times [DR] \times A^o \quad (7.2)$$

where $k_{DR} = 3.1 \times 10^9$ m^{-1} s^{-1} determined by pulse radiolysis studies; the rate constants are not influenced by [DR], provided that it is ≥ 2.8 mM [118].

If one is investigating the •OH scavenging activity of an unknown antioxidant compound, or a crude extract, then the results can be expressed in reference to inhibition of deoxyribose degradation as follows [119]:

$$\%\ \textbf{Inhibition} = \frac{\left(A532\ \textbf{Control} - A532\ \text{Sample}\right)}{A532\ \text{Control}} \quad (7.3)$$

where $A532$ **Control** = absorbance in the absence of the sample antioxidant under study; A_{532} Sample = absorbance in the presence of the sample antioxidant compound.

Following the initial description of the deoxyribose •OH scavenging activity methodology, further attention was paid to the role of the EDTA-$Fe^{2+/3+}$ chelation complex and how it may impact the degradation of deoxyribose [120]. A multidentate ligand, such as EDTA, binds $Fe^{2+/3+}$ in solution away from the substrate, resulting in the 'non-site specific' •OH degradation of deoxyribose; on the other hand, Aruoma and coworkers [120] demonstrated that deoxyribose itself can act as a bidentate ligand of Fe^{3+}, thus, $Fe^{2+/3+}$ can bind directly to deoxyribose, resulting in 'site-specific' •OH-mediated degradation of deoxyribose in the absence of EDTA.

7.4.3.1.6 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH•) Stable Free Radical Scavenging Assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH•; also known as α,α-diphenyl-β-picrylhydrazyl, 2,2-diphenyl-1-picrylhydrazyl or 2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl) assay originally described by Blois [121] was designed to take advantage of a common electron spin resonance reagent, a stable free radical with an odd, unpaired valence electron to study antioxidant activity. With its odd electron, DPPH• can be stabilized by accepting an electron or hydrogen radical from an antioxidant molecule such as a sulfhydryl group [121]:

DPPH• is known for its deep violet colour and strong absorbance at 517 nm when dissolved in ethanol at concentrations

between 1 mM to 22.5 μM [121, 122, 124]; this absorbance decreases with the decolourization of DPPH• which accompanies the pairing of the lone electron. The A_{517} of DPPH• is stable between pH 5.0 and 6.5, but sensitive to highly alkaline conditions which can be buffered by acetate [121, 124]. The wide range of DPPH• concentrations used in the literature is no doubt related to the limited solubility of this stable free radical (Table 7.1). Moreover, studies using DPPH• have varied widely not only in the solvent used to dissolve the stable free radical, but also the wavelength used to monitor the decolourization of the stable free radical [119, 121, 122, 124–128]. For example, studies monitoring the A_{517} decolourization of DPPH•-sample solutions used ethanol as originally recommended by Blois [121], but also diluted ethanol, methanol, diluted methanol and dimethylsulfoxide (DMSO). Interestingly, when Sharma and Bhat [124] measured the A_{517} of DPPH• over a wide range of concentrations (approx. 10–250 μM) using different solvent systems, the A_{517} varied as follows: 60% methanol was slightly > methanol > ethanol, thus, 517 nm may not have been the optimal wavelength to monitor the decolourization of DPPH• in differing solvents. The choice of solvent may also have been influenced by the solubility of the antioxidant compounds or extracts under evaluation, since the DPPH• stable free radical scavenging methodology can be used to study both polar and nonpolar antioxidants such as ascorbic acid and butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT), respectively. For example, methanol is the preferred solvent for non-glycosylated flavonoids which are considerably less water-soluble than the respective glycones [128]; whereas, DMSO is noted to be highly polar, very stable, miscible with most common organic solvents and dissolves most aromatic and unsaturated hydrocarbons, organic nitrogen compounds, organo-sulfur compounds and many inorganic salts, but does not dissolve saturated hydrocarbons.

The percentages of DPPH• remaining at steady state are then plotted against the corresponding [antioxidant] resulting in a graph that allows the effective concentration for 50% reduction in DPPH• (EC_{50}) to be calculated [125]. On the other hand, many investigators have chosen a single time point to quantify the DPPH• stable free radical scavenging efficacy of the antioxidants under study, with the most common choice as 30 min (varies from 20 to 60 or 90 min) and compared these values with a solvent control to express the inhibition of the DPPH• stable free radical [119]:

Table 7.1 Survey of DPPH• stable free radical scavenging methodologies.

Reference	[DPPH•] (μM)	Solvent[a]	(nm)	Temp[b]. (°C)	Quantitation of DPPH• scavenging activity.
[121]	500	ethanol	517	RT	A_{517}, incubation time not identified;
[126]	229	50% ethanol	528	RT	A_{528} monitored from 0 to 20 min.;
[125]	60	methanol	515	RT	monitor decease in A_{515} until a plateau (steady state) is reached, 1 min. to 6 hr.;
[122]	1000	methanol	517	RT	A_{517} after 30 min. incubation of sample;
[129]	200	methanol	517	RT	A_{517} after 30 min. incubation of sample;
[127]	30	DMSO	517	RT	A_{517} after 60, 90 min. incubation of sample;
[119]	100	ethanol	519	RT	A_{519} after 20 min. incubation of sample;
[128]	200	50% ethanol	523	RT	monitor decease in A_{523} until a plateau (steady state) is reached, 1 min. to 2 hr.;

[a] DMSO = dimethylsulfoxide;

[b] RT = room temperature.

$$\%\ \text{Inhibition} = \left(\left(\text{A"519 Control - "A"519 Sample}\right)\text{"}\right) \text{/"519 Control"} \times 100\right) \quad (7.4)$$

However, unless the antioxidants are screened and identified as having rapid or intermediate kinetics, the stable free radical scavenging activity can be underestimated [124, 125]. Indeed, when the DPPH• stable free radical scavenging activities were determined (using methanol as the solvent) for the lipid soluble antioxidant BHT, Brand-Williams and coworkers [125] observed EC_{50} values of 0.943 mol/L after 30 min incubation reaction time and 0.189 mol/L after 240 min, after a steady state plateau had been reached, a five-fold difference in antioxidant power. On the other hand, the difficulties in the interpretation of data from different studies is demonstrated despite the differences

7.4.3.1.7 Azo Dyes as Sources of Stable Free Radicals in Antioxidant Assays

Azo compounds, or dyes, are distinguished by containing an azo group –N=N- within their structure and comprise a large class of synthetic organic dyes such as Congo red and Tartrazine; approximately 60–70% of dyes used in the food and textile industries are azo dyes. Azo compounds are also regularly used as free radical initiators in the study of antioxidant compounds, and particularly the quantitation of lipid peroxidation *in vitro* and *vivo*, due to the predictable thermal decomposition of these compounds to yield N_2 and two carbon radicals, R• [130].

These radicals may then either react with each other to yield a stable non-radical end product (R-R), or react with molecular O_2 to yield peroxyl radicals, ROO• which can then participate in the peroxidation of a polyunsaturated lipid emulsion model system. The structure or composition of R will determine not only the solubility of the azo compound, but also the kinetics of the decomposition. Two commonly used hydrophilic radical initiators used in the recent literature are, 2, 2′-azo-bis-(2-amidipropropane hydrochloride) (ABAP) and 2, 2′-azo-bis(2-amidinopropane) dihydrochloride (AAPH). ABAP and AAPH are the same chemical, just differing with one HCl moiety in the former and two HCl moieties present in the latter. Due to its polarity, AAPH generates its radicals in the aqueous region of an oil-in-water emulsion used in studying lipid peroxidation; whereas, 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) is a lipophilic radical initiator which generates its

radicals within the lipid regions of emulsion, micelle droplets or membranes [130–132]. More recently, Noguchi and coworkers [131] described a novel lipophilic azo free radical initiator, 2, 2′-azobis (4-methoxy-2,3-dimethylvaleronitrile) (MeO-AMVN). The decomposition of azo radical initiators is a function of mainly temperature, and solvent and pH to a lesser extent [130]. For example, at 37°C and neutral pH, the $T_{1/2}$ of AAPH is 175 h [130]; whereas, at 37°C and acetonitrile as the solvent, the $T_{1/2}$ of AMVN is 100 and that of MeO-AMVN is 6.06 h [131, 133]. Therefore, the rate of radicals (R_i) generation would be constant over the short term in the case of an accelerated lipid oxidation model using a free radical initiator and elevated temperature of incubation [132, 134–136]. The rate of generation of AAPH radicals (R_i) at 37°C, neutral pH is as follows [130]:

$$R_i\ (sec^{-1}) = 1.36 \times 10^{-6}\ mol/liter/sec\ [AAPH] \qquad (7.5)$$

where [AAPH] is expressed in mol/liter, and the total amounts of free radicals released calculated from:

$$\text{Total amount of free radical released} = 1.36 \times 10^{-6}\ [AAPH] \times t \qquad (7.6)$$

where t = incubation time in seconds; the rate for AMVN radicals at 37°C in acetonitrile [133]:

$$R_i\ (sec^{-1}) = 3.88 \times 10^{-6}\ mol/liter/sec\ [AMVN] \qquad (7.7)$$

the rate for MeO-AMVN radicals at 37°C in acetonitrile [131]:

$$R_i\ (sec^{-1}) = 3.18 \times 10^{-5}\ mol/liter/sec\ [MeO\text{-}AMVN] \qquad (7.8)$$

Thus, it is possible to calculate the total amounts of azo-derived free radicals released, and thereby the corresponding concentrations, during *in vitro* or *ex vivo* (e.g. red blood cell suspensions or hepatic microsomal preparations) model system studies [130,132].

The solubility of the azo free radical initiator used is very important with respect to the polarity of the antioxidant(s) under study as well as the composition of the model system [130, 132]. For example, Niki [130] proposed that if AAPH is used to generate free radicals in a liposome system with a lipophilic antioxidant such as α-tocopherol, care should be taken to sonicate multilamellar liposomes to yield unilamellar liposomes to facilitate the interaction of AAPH radicals with the antioxidant, which otherwise would not be possible if the antioxidant was located within the inner

membranes of a multilamellar system. Similarly, Yuan and coworkers [132] studying the antioxidant activity of red algal *Palmaria palmata* (dulse) extracts, observed a protective effect of extracts on the production of 2-thiobarbituric acid reactive substances (TBARS; Table 7.2) in linoleic acid emulsions when AAPH was the free radical initiator, but lacked dose response. On the other hand, there was an absence of a protective effect of dulse extracts when AMVN was used as the free radical initiator. The lack of a dose response was attributed to the 'polar paradox' where polar compounds exhibit weak antioxidant activity in emulsions due to the dilution of antioxidants in the aqueous phase [132, 137]; whereas, the lack of a protective effect of dulse extracts in the presence of AMVN-induced lipid peroxidation, *versus* that with AAPH, could be associated with the localisation of the free radicals within the lipid phase of the emulsion which would not be in contact with the aqueous dulse extract constituents and, thereby, lipid peroxidation would not be inhibited.

7.4.3.1.8 Oxygen Radical Absorbance Capacity (ORAC) Assay

When originally developed, the ORAC assay was designed to evaluate the protective effect of antioxidant compounds against reactive oxygen species-mediated damage to the fluorescent indicator R- or β-phycoerythrin (PE); free radicals were derived from AAPH or •OH in the presence of $Cu^{1+/2+}$ and ascorbic acid [139]. However, despite the linear, zero order kinetics exhibited by PE in the ORAC assay, the natural variability of this reagent and its instability to photo-bleaching (necessitating making a fresh PE solution daily) and interaction with polyphenols led researchers to look for an alternate fluorescent indicator. Fluorescein was subsequently demonstrated to not only have excellent photostability within assay conditions, but also not to have any interactions with antioxidant molecules, such as polyphenols [139]. Thus, fluorescein solutions in 75 mM phosphate buffer, pH 7.4–7.0 can be stored at 4°C for 4 weeks, or -70°C for several months when protected from light exposure [139–141]. The $ORAC_{FL}$ assay has gained great acceptance amongst the food science, functional food and nutraceutical research community, and indeed by marketers of such foods, due to its utility in analyzing multiple samples quickly using 96-well microplates. Moreover, the $ORAC_{FL}$ assay allows for both automated (robotic) reagent handling, as well as manual procedures using multichannel pipettors [139]. The one

Table 7.2 Summary of *in vitro* and *ex vivo* azo compound antioxidant methodologies.

Azo compound[a]	[Azo] mM	Model system[b]	References
AAPH	25	Microsomal suspension, 37°C	[135]
	100	10% red blood cell suspension, pH 7.4, 37°C	[134]
	10	74 mM methyl linoleate micelles, 37°C	[131]
	10	10% (v/v) linoleic acid, 5 mM phosphate buffer, pH 7.4, emulsion, 37°C	[132]
	25–100	10% rabbit red blood cell suspension in 125 mM NaCl, 10 mM phosphate buffer, pH 7.3	[130]
	10	methyl linoleate emulsion, soybean phospha-tidylcholine liposomes, or red blood cell ghosts, 37°C	[130]
AMVN	2.0	7.4 mM methyl linoleate in acetonitrile, 37°C, with fluorescent probe BODIPY	[133]
	0.8	25 mM methyl linoleate in acetonitrile, 37°C	[138]
	10	10% (v/v) linoleic acid, 5 mM phosphate buffer, pH 7.4, emulsion, 37°C	[132]
	1.0	5.1 mM soybean phosphatidylcholine liposomes, 3.0 μM α-tocopherol, 5 mM phosphate buffer, pH 7.4 with 0.1 M NaCl	[130]
MeO-AMVN	0.05	453 mM methyl linoleate, 10 μM α-toco-pherol in acetonitrile, 37°C	[131]

[a] AAPH = 2,2′-azo-bis(2-amidinopropane) dihydrochloride; AMVN = 2,2′-azobis(2,4-dimethylvaleronitrile); MeO-AMVN = 2,2′-azobis (4-methoxy-2,3-dimethylvaleronitrile);

[b] BODIPY = 4,4-difluoro-5-(4-phenyl-1,3 butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid;

impediment to the adoption of this antioxidant methodology is the initial cost of a fluorescent microplate reader with a shaker and incubation to analyze the samples.

The $ORAC_{FL}$ assay is based on quantitation of antioxidant activity from the area under the curve (AUC) calculated from the decay in fluorescence intensity when fluorescein is degraded by AAPH-derived peroxy radicals.

$$AUC = 1 + \sum_{i=1}^{i=80} \frac{\int_i}{\int_0} \tag{7.9}$$

where $\int_0$ = initial fluorescence at time 0 min and $\int_i$ = fluorescence reading at time i min [139]. Thus, this assay takes advantage of the generation of peroxyl radicals derived from this azo compound at a known and constant rate at 37°C in a model system. Quantitation of $ORAC_{FL}$ values are expressed relative to the reference antioxidant, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), a water soluble analogue of vitamin E. Typical assay conditions consist of (final concentrations in 200 µL volume): 75 mM phosphate buffer, pH 7.4–7.0 with 60–70 nM fluorescein, in the presence or absence of sample antioxidant, pre-incubated at 37°C, 15 min; followed by the addition of 12 mM AAPH and fluorescence (excitation at 485, emission at 527 nm) recorded every minute up to 60–80 min with shaking between readings [139–141]. $ORAC_{FL}$ activity is quantified as µmol Trolox equivalents/g or mL sample as necessary; Trolox standards used range from 0.5 to 8 µM (final concentrations).

7.4.3.1.9 Total Radical-Trapping Antioxidant Parameter (TRAP) Assay

The original total peroxyl radical trapping antioxidant parameter (TRAP) methodology described by Wayner and coworkers [142] was designed to assess the capacity of plasma antioxidant constituents to quench or trap azo compound-derived peroxy radicals from the thermal decomposition of ABAP or AAPH as discussed above (Table 7.3). Briefly, the TRAP assay conditions comprised monitoring the uptake of O_2, using an oxygen electrode, by a test sample containing 3mL 3.8 mM ABAP in 5 mM phosphate buffered saline, pH 8+ 30 µL plasma, incubated at 37°C. The TRAP value is calculated as follows:

$$\text{TRAP}\left(\mu\text{mol peroxyl radicals}\frac{\text{trapped}}{\text{L}}\text{plasma}\right) = R_i\,(\text{plasma}) \tag{7.10}$$

where plasma = induction period, the length of time that O_2 uptake is inhibited by the plasma and R_i is obtained from the addition of a known concentration of Trolox (5.7 nmol) to the incubated sample after the endogenous plasma antioxidants have been exhausted.

$$R_i = n[\text{Trolox}] \div \tau_{\text{Trolox}} \tag{7.11}$$

where n = 2, the stoichiometric factor represented by the number of peroxyl radicals trapped per molecule of Trolox and τ_{Trolox} = the length of time that O_2 uptake is inhibited by the addition of Trolox to the sample, a second induction period [142]. Total incubation time for the TRAP assay can be up to 120 min, which may exceed the stability of the oxygen electrode [63, 143, 144]. It is noteworthy that the TRAP values for fresh plasma collected with Na_2EDTA were very similar to the ferric reducing ability of plasma (FRAP) values discussed below, ranging between 806–1115 µM (Table 7.3).

Subsequent modifications to the TRAP assay methodology moved away from monitoring O_2 uptake in favour of using fluorescent indicators and monitoring the degradation of these molecules, such as PE [143], as in the ORAC assay above; these workers added 8 µL plasma to 2mL 15 nM PE in 75 mM phosphate buffer, pH 7 with incubation at 37°C, 5 min., followed by the addition of ABAP 4mM, final concentration (Table 7.3). However, as above, the inherent variability of this naturally occurring pigment and its lack of photostability led to its replacement by other indicators such as the nonfluorescent 2,7-dichlorofluorescin-diacetate (DCFH-DA [145]). When DCFH-DA is reacted with AAPH at RT, it is converted to the highly fluorescent dichlorofluorescein (DCF; ex. 480 nm, em. 526 nm) which also exhibits A_{504} allowing the use of a fluorometer or spectrophotometer. Plasma samples were diluted to 1% in phosphate-buffered saline with the addition of DCFH-DA 14 µM (final concentration) and the reaction started with AAPH 56 mM (final concentration) and the fluorescence or A_{504} monitored over 100 min [145]. In contrast to the ORAC assay, monitoring DCF fluorescence results in an initial lag phase whilst the endogenous antioxidants are depleted, followed by a rapid increase in fluorescence, representing a propagation phase. There is a second lag phase attributed to the effects of the addition of Trolox as an internal standard, and another propagation phase after the Trolox has been depleted. Valkonen and Kuusi [145] reported lag phases, plasma = 15 min,

Trolox = 19.5 min and TRAP values of 1292 µM for fresh plasma (Table 7.3), whereas after storage at -80°C, 2 mo, the corresponding values were 16.5 min, 23 min and 1205 µM, respectively.

4.3.1.10. ABTS•+ Radical Cation Scavenging Activity

The radical cation form of 2,2′-azino-bis-(3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS) is generated by oxidizing ABTS with potassium persulfate to form ABTS•+; a blue-green chromophore with absorbance maxima at 415, 645, 734 and 815 nm [151, 152]. Similar to the DPPH• stable free radical scavenger assay above, the evaluation of potential antioxidant activity using ABTS•+ involves the decolourisation of the cation radical solution by the antioxidant compound donating an electron or hydrogen atom. The original version of this radical cation antioxidant assay involved the activation of metmyoglobin by H_2O_2 in the presence of ABTS in order to generate ABTS•+ in the presence or absence of antioxidant compounds; however, potential interference from antioxidants with rapid kinetics able to reduce the ferryl myoglobin radical was a criticism of this methodology [152]. Thus, the ABTS•+ free radical scavenging assay was altered to incubate test compounds with preformed radical cation molecules as opposed to generating the radical cations in the presence of the test antioxidants [151, 152]. Briefly, typical assay conditions consist of generating the ABTS•+ radical cation by reacting 7 mM ABTS with 2.45 mM potassium persulfate (final concentration) overnight (12–16 h) in the dark, at room temperature, followed by dilution with ethanol to achieve an A_{734} = 0.70 at 30°C in a temperature controlled spectrophotometer [128, 152]. Free radical scavenging by hydrophilic or lipophilic antioxidants or extracts can then be monitored by mixing 1mL diluted ABTS•+ radical cation with 10 µL of test antioxidant and monitoring the A_{734} at 0, 0.5, 1 min and again at 5 min intervals until a steady state plateau is achieved [128, 151, 152].

$$\% \textbf{ Inhibition} = \frac{\left(\text{A734 Control} - \text{A734 Sample}\right)}{A734 \text{ Control}} \quad (7.12)$$

Sample antioxidants can then be tested for ABTS•+ radical cation scavenging efficacy including phenolics, flavonoids and hydroxycinnamates solubilised in ethanol; anthocyanidins in acidic

ethanol, pH 1.3; carotenoids (lycopene and β-carotene) dissolved in dichloromethane; α-tocopherol in ethanol and plasma antioxidants diluted with water [151, 152]. When Hu and Kitts [153] evaluated the ABTS$^{\bullet+}$ radical cation scavenging activities of chloroform and methanol Echinacea root extracts (*E. purpurea, E. angustifolia and E. pallida*), the antioxidant activity of the methanol extracts (11.0–63.8% inhibition) was 3.4 to 20-fold greater than that of the chloroform extracts (1.8–3.2% inhibition). Interestingly, the ABTS$^{\bullet+}$ radical cation scavenging EC_{50} values for L-ascorbic acid, BHA and the red marine alga *Palmaria palmata* observed by Yuan and coworkers [128] were relatively similar to the corresponding results obtained for these antioxidants in the DPPH• free radical scavenger assay for both kinetics (rapid *versus* slow) as well as antioxidant efficacy.

7.4.3.1.11 Ferric Reducing Ability of Plasma (FRAP) Assay

The ferric reducing ability of plasma (FRAP) assay was designed as a simple, inexpensive method to quantify the collective non-enzymatic antioxidant capacity of biological fluids such as plasma, saliva, tears, urine and cerebrospinal fluid [154, 155]. It was proposed that this assay could evaluate the combined effect of plasma antioxidant constituents such as glutathione (GSH), albumin, α-tocopherol, uric acid, bilirubin, ascorbate, etc.; thus, the FRAP assay could directly measure the 'total antioxidant power' of a complex mixture with potential synergistic effects which would not be evident when assayed as single components [155]. The FRAP assay is based on the single electron transfer by an antioxidant to reduce the ferric to ferrous ion; when the ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex is reduced to the ferrous counterpart, the complex absorbs at 593 nm with an intense blue colour.

Briefly, the original FRAP reagent consisted of (final concentrations): 250 mM acetate buffer, pH 3.6; 0.833 mM 2,4,6-tripyridyl-*s*-triazine (TPTZ) in 3.33 mM HCl, and 1.67 mM $FeCl_3 \cdot 6H_2O$ (freshly made up in degassed/deaerated water); 300 µL reagent is warmed to 37°C prior to addition of 10µL sample and 30 µL H_20 and A_{593} monitored after 0.5 sec. and then every 15 sec., up to 8 min, against a reagent blank [154]. The FRAP activity is calculated as follows:

$$\text{FRAP activity of sample}\,(\mu\text{M}) = \frac{\left(A593\,0 - \text{x min. Sample}\right)}{A593\,0 - \text{x min. Std}} \times \text{FRAP value of Std}\,(\mu\text{M}) \quad (7.13)$$

Table 7.3 Summary of TRAP assay reagents and methodologies.

Sample[a]	Peroxyl radical source[b]	Indicator monitored[c]	TRAP values[d]	References
Plasma	ABAP	O_2 consumption by O_2 electrode;	806–1115 μM	[142]
Plasma	ABAP	R- or β-phycoerythrin (PE)	850–1530 μM	[143]
Plasma	AAPH	DCFH- DA	1292 μM	[145]
Plasma;	ABAP	Luminol	988–1288 μM;	[144]
CSF			240 μM	
LDL	AMVN	Luminol	26.5 μmol/mmol P_i	[146]
Berry extracts	AAPH	Luminol	2132–4051 μmol TE/g extract	[147]
Citrus fruit flesh;	ABAP	Luminol	1111–4480 nmol/mL;	[148]
citrus peel			1667–6720 nmol/mL	
Root and leafy	AAPH	Luminol	1.0–68.1 μmol	[149]

Sample[a]	Peroxyl radical source[b]	Indicator monitored[c]	TRAP values[d]	References
vegetables			TE/g fresh wt.	
Black, green tea	ABAP	R- or β-phycoerythrin (PE)	3.5, 17.8 mM	[150]
White, red wine			1.9, 40 mM	
Plasma			1308–1343 µM	
Plasma-black tea			1761 µM	
Plasma-green tea			1826 µM	
Plasma-red wine			1395 µM	

[a] CSF = cerebrospinal fluid, LDL = low density lipoprotein; Berry extracts = black current, blackberry, blueberry, elderberry and chokeberry (from lowest to highest TRAP value); Citrus fruit and peel = grapefruit, orange and lemon (lowest to highest); Red and white wine were alcohol-free;

[b] ABAP = 2,2′-azo-bis(2-amidinopropane) hydrochloride; AAPH = 2,2′-azo-bis(2-amidinopropane) dihydrochloride; AMVN = 2,2′-azobis(2,4-dimethylvaleronitrile);

[c] DCFH-DA = 2,7-dichlorofluorescin-diacetate; Luminol = 5-amino-2,3-dihydro-1,4-phthalazinedione;

[d] P_i = inorganic phosphorus; TE = Trolox equivalents.

where x min = final reading when A_{593} has reached a steady state plateau; x = 4 min when the assay is run at 37°C, and; x = 6 min at RT. The FRAP value of the Standard is determined using $FeSO_4 \cdot 7H_2O$ in the range 100–1000 µM; the solvent corrected A_{593} of Fe^{2+} 100 µM = a FRAP value of 100 µM [155]. The value for x min may vary depending on the kinetics of the sample antioxidant being evaluated; for example, L-ascorbic acid and α-tocopherol (solubilised in ethanol) exhibit rapid kinetics with a steady state plateau reached within 1 min, uric acid reaches a steady state plateau after 3 min, whereas, bilirubin does not reach a steady state plateau within the 8 min assay [154]. The importance of assay temperature is demonstrated in particular with uric acid which exhibits slower reaction kinetics at RT [155].

When Benzie and Strain [154, 155] determined the FRAP activity of freshly collected, fasting human plasma containing EDTA, the values ranged between 612 to 1634 µM. However, the FRAP value for the corresponding serum was only 40% of that for plasma; which was partly attributed to the contribution from albumin in the FRAP assay (plasma contains approx. 700 µmol/L albumin [154]. When albumin was analysed on its own, the A_{593} exhibited a slow increase over time associated with the fact that while albumin remains soluble in solution at the low pH of the assay conditions, its low reactivity in the FRAP assay is due to the low pH effects on protein thiol groups [154]. On the other hand, plasma FRAP values have been positively correlated with plasma uric acid contents [154].

The FRAP values of extracts from blueberries, blackberries, raspberries and strawberries varied considerably with the extraction solvent: 70:30 acetone: water extracts > 70:30 ethanol: water $\geq$ water alone when determined at RT; differences between berries: blackberries (68 µmoles) > blueberries (47 µmoles) > raspberries = strawberries (40 µmoles) were attributed to the amounts of polyphenols extracted with the solvent mixtures [156]. However, differences in anthocyanidin composition and chemistry at pH 3.6 (related to the flavylium cation) cannot be discounted when analysing these data. It is noteworthy that these workers demonstrated that even after 250 min incubation, the 70:30 acetone: water berry extracts had not reached a steady state plateau at A_{593}, which may be due to the lower incubation temperature used therein. On the other hand, Szeto and coworkers [157] reported that an aqueous extract of fresh strawberries exhibited the greatest FRAP value (15940 µmoles/kg fresh wt) compared to various citrus fruits, red

and green grapes; moreover, this activity was closely related to the ascorbic acid content of these fruits. The most important difference between these two studies is that Henríquez and coworkers [156] extracted frozen (-20°C) fruit, which would have undergone oxidation of ascorbate during storage. Thus, not only is extraction solvent crucial to FRAP value results, but also, storage or freshness of the samples given that many antioxidant molecules are susceptible to oxidation or degradation.

On the other hand, a modification to the FRAP assay, named the FRASC assay, allows the dual measurements of ascorbic acid content and FRAP activity in one test system. For the FRASC assay, one sample aliquot is treated with ascorbate oxidase 40 µL of ascorbate oxidase (4 U/mL is added to 100 µL sample) to degrade the ascorbic acid while its pair is left untreated [158]. The remaining reagents and methods for the FRASC assay are the same as for FRAP analysis. The ascorbic acid (AA) concentration can be calculated according to:

$$0 - 1 \text{ min AA related } A_{593} = (0 \text{ -1 min }) A_{593} \text{ Sample} - \text{ao}) - (0\text{–}1 \text{ min}) A_{593} \text{ Sample} + a_0) \quad (7.14)$$

where - ao = paired water, and + ao = ascorbate oxidase diluted samples; and

$$[\text{AA}](\mu\text{M}) = [\text{AA}]\,\text{Std}\,(\mu\text{M}) \times \frac{0-1\,\text{min. AA related}\left(A593\,\text{Sample}\right)}{0-1\,\text{min. AA related}\left(A593\,\text{Std}\right)} \quad (7.15)$$

where the appropriate AA standard concentrations are chosen [155, 158]. Thus, Benzie and Strain [158] reported FRAP values of 1018 µM and ascorbic acid levels of 51 µM for fresh plasma with EDTA; whereas, aged plasma (-70°C, 3 mo) did not contain any ascorbic acid.

7.4.3.1.12 Other Assays–Methods Based on the Chemiluminescence (CL) of Luminal

The main principle of these methods is based on the ability of luminol and related compounds to luminescence under the flux of free radicals (chemiluminescence, CL) [68]. CL is brought about due to a reaction of free radical derived from luminol with active free

radicals. CL can be easily recorded. The addition of an antioxidant compound, being a scavenger of an active free radical, results in CL quenching, commonly with a pronounced induction period [68]. The quantity of the tested antioxidant can be estimated from the duration of tIND. As a rule, antioxidant activity is given in Trolox equivalents. The attractive feature of CL methods is their productivity; commonly, one run normally takes a few minutes only; in addition, the assay can be easily automated. As for shortcomings of this group of methods, first of all, the mechanism for chemical processes resulting in CL is not known in detail. The latter may create problems with interpreting data obtained. Different versions of this method differ in the type of active free radical produced and the way of free radical production as well as in details of the protocol. While the majority of assays have been developed for testing biologically relevant samples, they can be easily applied for food testing) [68]. Parejo, Codina, Petrakis, and Kefalas [159] proposed inducing CL by reaction of Co(2+) chelated by EDTA with H_2O_2. Although the authors suggested HO as an active free radical, which attacks luminol, it is more realistic that $O^{\cdot-}_2$ plays this role. The method is well-instrumented and computerized. The capability of the method was demonstrated by the example of testing several natural products including wines, tea, and medicinal herb extracts. The evident advantage of the method is its very high productivity: the procedure commonly takes a couple of minutes only. At the same time, the kinetic theory of the process underlying the assay has not been thoroughly investigated [68].

7.5 Problems in Comparing Various Methods of Antioxidant Activity and Discrepancies over Their Measurement

Antioxidant content of food samples (fresh or processed) may be characterized by two independent parameters: antioxidant capacity and reactivity [68]. For individual antioxidants, this corresponds to the stoichiometric coefficient and the rate constant of reaction between antioxidants and highly reactive free radicals. There is no single robust answer to the question which index of antioxidant activity is more relevant. The main attention is currently paid to

determining antioxidant capacity. The absolute majority of the recently developed methods are designed to solve this major problem. Admittedly, the reactivity of food samples may be of interest under certain conditions. Meanwhile, the information on the reactivity of food and individual natural polyphenols is still rather poor and conflicting [68].

Indirect methods (DPPH, ABTS) are used more frequently than direct methods (competitive crocin bleaching, competitive β-carotene bleaching). The question now arises which of the methods, direct or indirect, is better in principle. Each method has both advantages and disadvantages. The direct methods are more adequate in principle, especially those based on the model of the controlled chain reaction. Besides, they are commonly more sensitive. The disadvantage of the direct methods is that most are time-consuming and their application requires significant experience in chemical kinetics [68]. Consequently, direct methods are commonly not adequately suitable for routine testing of natural products.

As a rule, well-developed indirect methods, such as the DPPH and ABTS assays, are more productive and easier in handling [68]. The crucial point concerning the application of indirect methods is their informative capability. The indirect methods commonly provide information on the capability of natural products to scavenge stable free radicals, e.g., DPPH and ABTS+. Undoubtedly, the best indirect methods as well as the Folin–Ciocalteu test allow the estimation of antioxidant activity to the first approximation [68]. However, it is questionable whether the raw data obtained with indirect methods give quantitative information on the capability of natural products to inhibit oxidative processes.

Various assays have been introduced to measure antioxidant activity of foods and biological samples lately. It describes the ability of redox molecules in foods and biological systems to scavenge free radicals.

It has been previously reported that antioxidant capacity determined by *in vitro* assays differs [160–163]. Ou *et al.* [160] conducted a large scale vegetable analysis using two different *in vitro* assays, FRAP and ORAC, and obtained very different antioxidant capacities from these methods. In their study antioxidant capacities determined by FRAP and ORAC assays were only weakly correlated. Pellegrini *et al.* [164] reported that rankings of several fruits, vegetables and beverages differed based on antioxidant capacity

measured by FRAP and ABTS assays suggesting that caution should be exercised when interpreting antioxidant capacities from different assays.

Xu and Chang [165] studied the effect of soaking, boiling, and steaming on antioxidant activities of cool season food legumes by two different methods (FRAP and ORAC). As compared to original unprocessed legumes, all processing steps caused significant ($p < 0.05$) decreases in total phenolic content, DPPH and ORAC values in all tested cool season food legumes (green pea, yellow pea, chickpea and lentil). In contrast, oxygen radical absorbance capacities increased with increasing pressure in both pressure boiling and pressure steaming treatments. TPC and DPPH were not parallel with ORAC in cases of pressure boiling and pressure steaming treatments. This phenomenon could be attributed to the increases or the formation (after high pressure heat treatments) of specific compounds, which could provide more hydrogen atom during oxidation–reduction reaction. Dudonne *et al.* [166] reported a strong positive correlation between ABTS and DPPH assays with a Pearson correlation coefficient of $r = 0.906$ when used for 30 aqueous plant extracts.

By definition, the antioxidant activity is the capability of a compound (composition) to inhibit oxidative degradation, e.g., lipid peroxidation. Phenolics are the main antioxidant components of foods [68]. Antioxidant activity of polyphenols is associated with various mechanisms of action, the elevated reactivity of phenolics towards active free radicals is considered as the most common principle mechanism. The authors would like to distinguish between the antioxidant activity and the reactivity. The antioxidant activity gives the information about the duration of antioxidative action; the reactivity characterizes the starting dynamics of antioxidation at a certain concentration of an antioxidant or complex antioxidant mixture [68].

It should be remembered that the abovementioned methods are intended for the determination of antioxidant activity of food sample per se, i.e., the antioxidative potential of food. As for the antioxidative action of food substituents in real biological systems, this will also depend on their bioavailability and food antioxidants metabolism. The authors also strictly recommend complete standardization of antioxidant assays as the results in the above studies can be confusing.

7.6 Methods for Antioxidant Phytochemical Analysis

7.6.1 Spectrophotometer

Most carotenoids exhibit maximum absorption in the visible region of the spectrum, between 400 and 500 nm. Because they obey the Beer-Lambert's law (i.e., linear absorbance proportional to concentration), absorbance measurements can be used to quantify the concentration of a pure (standard) carotenoid or estimate the total carotenoid concentration in a mixture or extract of carotenoids in a sample. However, to estimate the presence of total carotenids in food products they have to be generally extracted by a solvent which in most of the cases is a non-polar liquid such as hexane in combination with lesser non-polar solvents such as acetone, ethyl acetate, methanol, petroleum ether, etc. [167–170] (Table 7.4).

7.6.2 High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) is a powerful technique for the separation, identification and quantification of phenolic and carotenoid compounds in plant extracts.

7.6.2.1 Phenolics

This method typically utilizes the power of reversed phase C_{18} column along with the binary solvent system containing acidified water (solvent A) and organic solvents (solvent B) such as methanol and acetonitrile to separate the phenolics. The introduction of reversed-phase columns has considerably enhanced the HPLC separation of phenolic compounds [171]. Reversed-phase HPLC coupled with diode array detector (RP-HPLC-DAD) has been the dominating analytical tool in separation science due to its relatively low cost, easy procedure and flexibility to use in conjunction with various state-of-the-art techniques for detection and structural elucidation of the analytes, for example, mass spectrometry, tandem mass spectrometry and nuclear magnetic resonance. The major limitation of HPLC-DAD is relatively low sensitivity and inability to detect simultaneously eluted compounds. In addition, DAD detection is not sufficient to discriminate between compounds with similar spectroscopic

Table 7.4 Spectrophotometry conditions used for the determination of carotenoids.

SNo	Food material	Compound	Extraction solvents	Spectrophotometer conditions	References
1.	Red and yellow tomato puree	lycopene	a) hexane / acetone / ethanol (50/25/25, v/v/v) b) Acetone/petroleum ether	at 450 and 503 nm. Acetonitrile (35–77%) in methanol.	[167]
2.	Red and yellow tomato puree	β-carotene	a) hexane / acetone / ethanol (50/25/25, v/v/v) b) Acetone/petroleum ether	at 450 and 503 nm. Acetonitrile (35–77%) in methanol.	[167]
3.	Orange Juice	Total carotenoids	mixture hexane/ methanol/acetone (50:25:25, v/v/v, with 0.1% butylated hydroxytoluene)	at 420, 455, 515, 545 and 610 nm/ 420, 445, 510, 545 and 605 nm	[123]
4.	Carrot	Total carotenoid	Hexane/acetone	at 450 nm	[169]
5.	Spinach	Total carotenoid	methanol/ethyl acetate/ petroleum ether (1:1:1, v/v/v)	at 450nm, diethyl ether	[170]

characteristics and the lack of reference compounds for comparison makes mass spectrometry and NMR supporting techniques very useful in characterisation of polyphenols. A selection of RP-HPLC-DAD methods to analyze polyphenols in plant extracts has been summarized in Table 7.5. RP-HPLC-DAD is generally performed by gradient elution (with changes in the mobile phase strength resulting from variation in composition of the solvents in the course of a chromatographic run). However, isocratic elution (with a mobile phase having constant composition) is also applied in RP-HPLC-DAD of polyphenols [172]. Gradient elution is usually obtained by gradual addition of a high-elution-strength solvent to a low-elution-strength solvent. Gradient elution lowers the analysis time and improves the separation efficiency of the method. Gradient elution is particularly useful in analysing extracts containing multiple components with varying polarity. The physical properties of the packing materials and size of the columns are important parameters in HPLC. Minimisation of the sorbent particles increases in the specific surface area of the sorbents, and controlled porosity generally increase column efficiency and make separation even of fairly complex mixtures possible. For example, columns with a low particle diameter of 3 µm perform better than those with 5 µm particles. Given the same length of the columns, the column with 3 µm particles can generate similar resolution to the column with 5 µm particles in half the separation time. Columns with smaller particles are therefore frequently used in the hyphenated HPLC techniques where lower solvent-input volumes are required for higher sensitivity.

7.6.2.2 Carotenoids

Carotenoid analysis of food samples are mainly performed by HPLC. Reversed-phase separations (Table 7.6) have been widely used in the determination of this kind of compounds. Both isocratic (Table 7.6) have been employed. In general, with gradient-solvent methods, the resolution achieved is better than with isocratic systems. However, the former presents some drawbacks, such as a higher total analysis time because of the necessity to re-equilibrate the column after each injection which represents a serious problem for routine analysis [195]. An ultraviolet-visible (UV-Vis) detector is the most widely employed detector in HPLC analysis and, more recently, the photodiode array detector (DAD), which allows a continuous collection of spectrophotometric data during the analysis [196].

Table 7.5 HPLC methods for the separation of phenolic compounds.

Serial No.	Name of the plant	Category of polyphenols	Extraction solvent	LC condition used	Reference
1	Strawberries and black currant	Anthocyanins	70% (v/v) methanol and 0.5% (v/v) HCl in HPLC-grade water	Zorbax Eclipse XDB-C18 column of 250 mm × 4.6 mm diameter, 5 µm particle size Mobile phase: 1% (v/v) phosphoric acid and 10% (v/v) acetic acid in HPLC-grade water (A) and acetonitrile (B). Flow rate: 1 mL/min column temperature, 35°C, injection volume, 10 µL, UV–Vis photo diode array detection at 520 nm	[173]
2	Blood orange	Anthocyanin	Filtered (0.45 µm diameter PTFE syringe filters) juice	Zorbax SB C_{18}, 15 cm × 4.6 cm, pore size 5 µm column Mobile phase: acetonitrile (33 % v/v); methanol (11 % v/v) and acetic acid (56 % v/v) which was mixed with trichloroacetic acid (0.65 g) Flow rate: 1 mL/min Injection volume, 20 µL, UV–Vis photo diode array detection at 520 nm	[172]

Serial No.	Name of the plant	Category of polyphenols	Extraction solvent	LC condition used	Reference
3	Tomato, pomegranate, grape, black carrot and wine	Anthocyanin	Juices and wine were centrifuged (3000xg) and filtered through 0.2 µm PVDF syringe filter	Inertsil ODS-3 C_{18} column (3 µm particle size, 150 × 4.6 mm) Solution A was composed of water:acetonitrile:formic acid (85:10:5, v/v) (pH adjusted to 2.0 using HCl) and solution B was composed of water: acetonitrile: formic acid (35:10:55, v/v) (pH adjusted to 2.0 using HCl) Flow rate: 0.8 mL/min column temperature, 40°C, injection volume, 10 µL, UV–Vis photo diode array detection at 520 nm	[174]
4	Apple	Phenolic acids and flavonoids	ethanol	Ccolumn (Develosil, 150 × 4.6 mm, i.d.) and a security guard C_{18} (4 × 3.0 mm, i.d.) 2% (v/v) acetic acid in water (eluent A) and 0.5% (v/v) acetic acid and 49.5% (v/v) acetonitrile in water (eluent B) The flow rate and injection volume were 1.0 mL/min and 20 µL, respectively Detected at 290 nm	[175]

(Continued)

Table 7.5 (*Cont.*)

Serial No.	Name of the plant	Category of polyphenols	Extraction solvent	LC condition used	Reference
5	Pomegranate	Anthocyanins	acetone (100%)	C_{18} (5 µm) column (250 × 4.6 mm) (Phenomenex, Los Angeles, CA) with a C_{18} (5 µm) guard column (4 × 3 mm, 5 µm) (Phenomenex, Los Angeles, CA) eluents used were (A) 100% acetonitrile and (B) *O*-phosphoric acid, acetic acid, acetonitrile and water (1:10:5:84; v/v/v/v) with a flow rate of 1 ml min^{-1}. The sample injection volume was 50 µl, the column temperature was 25°C, and the detector was set at 520 nm	[176]
6	Peach	Phenolic acids and flavonoids	petroleum ether, hexane, ethyl ether and chloroform	alphaBond C18 125A column (4.6 × 250 mm, particle size 5 µm) mobile phases consisted of 2.0% acetic acid in distilled water (A) and acetonitrile (B). Flow rate: 1.0 mL/min Sample injection volumes were 20 µL. Compounds were detected at 280 nm	[177]

Serial No.	Name of the plant	Category of polyphenols	Extraction solvent	LC condition used	Reference
7	Lamiaceae herbs	Phenolic acids and flavonoids	Aqueous methanol (80%)	Nucleosil 100-C18 column (5 μm, 250mmx4 mm) with a Nucleosil 5 C18 guard column (5 μm, 4 mmx4 mm) (solution A, 2.5% formic acid, and solution B, 100% methanol) flow rate 0.8 mL/min, injection volume 20 μL. Detection at 280 nm for flavanones, flavanols, hydroxybenzoic acids, tannins, phenolic diterpenes, and volatile compounds, at 320 nm for hydroxycinnamic acids and flavones, and at 370 nm for flavonols.	[178]

(Continued)

Table 7.5 (*Cont.*)

Serial No.	Name of the plant	Category of polyphenols	Extraction solvent	LC condition used	Reference
8	Apricot	Phenolic acids and flavonoids	methanol/HCl (100:1, v/v) which contained 2% *t*BHQ	Pinnacle C-18 column (250 × 4.6 mm i.d., 5 µm) protected by a guard column Pinnacle C-18 (10 × 4.6 mm i.d., 5 µm) mobile phase A contained 3% acetic acid in water; solution B contained mixture of 3% acetic acid, 25% acetonitrile and 72% water column temperature, 20°C, injection volume, 20 µL, UV–Vis photo diode array detection at 278 nm flow rate: 1–1.2 mL min^{-1}	[179]
9	Wine	Flavonoids	Filtered and direct injection	Nucleosil 100 C_{18}, (250 × 4 mm, 5 µm) coupled to C_{18} column guard A: 2 mM $NH_4H_2PO_4$, adjusted to pH 2.6 with H_3PO_4; B: 20%A with acetonitrile; C: 0.2 M H_3PO_4 adjusted to pH 1.5 with ammonia; Flow rate: 0.7 mL/min column temperature, 25°C, injection volume, 20 µL, UV–Vis photo diode array detection at 280 nm	[180]

Serial No.	Name of the plant	Category of polyphenols	Extraction solvent	LC condition used	Reference
10	Apples, grapes, beans	Flavonoids	with 90% MeOH (apples, grapes) or 70% MeOH (beans)	Inertsil ODS –2 (150 × 4.6 mm, 5 µm) coupled with Opti-Guard PR C18 Violet A guard A: 5% acetonitrile in 0.025 M phosphate buffer pH 2.4; B: 25% acetonitrile in 0.025 M phosphate buffer pH 2.4 Flow rate: 1.0 mL/min column temperature, 30°C, injection volume, 10 µL, UV–Vis photo diode array detection at 270 nm	[181]
11	Red onions	Anthocyanins	MeOH containing 0.1% HCl	Prodigy ODS2 (250 mm × 4.6 mm, 5 µm) A: 10% formic acid in H_2O (v/v); B: MeOH–H_2O–formic acid (50:40:10, v/v/v); Flow rate: 1.0 mL/min column temperature, 30°C, UV–Vis photo diode array detection at 520 nm	[182]

(Continued)

Table 7.5 (*Cont.*)

Serial No.	Name of the plant	Category of polyphenols	Extraction solvent	LC condition used	Reference
12	Billberry	Anthocyanin	50%MeOH containing 2%HCl	Ultrasphere ODS column (250 mm × 4.6 mm, 5 µm) A: 0.4%TFA in H_2O; B: 0.4% TFA in acetonitrile; JAFC	[183]
13	Spinach	Flavones and flavonols	40% MeOH	Phenomenex Luna phenyl-hexyl column (250 mm × 4.6 mm, 5 µm) coupled with security guard column Phenomenex C_{18} ODS (4 mm × 3 mm) A: H_2O/MeOH/formic acid (69:30:1); B: MeOH Flow rate: 0.7 mL/min Injection volume, 10 µL, UV–Vis photo diode array detection at 340 nm	[184]
14	Buckwheat	Flavones and flavonols	80% MeOH	Capcell Pak C_{18}-SG 120, column, (100 mm × 4.6 mm, 3 µm) A: MeOH–H_2O–acetic acid (13:36:1, v/v/v); B: MeOH–H_2O–acetic acid (73:25:2, v/v/v); Flow rate: 0.5 mL/min column temperature, 40°C, UV–Vis photo diode array detection at 350 nm	[185]

Serial No.	Name of the plant	Category of polyphenols	Extraction solvent	LC condition used	Reference
15	Tomatoes, onions, lettuce celery	Flavones and flavonols	1.2 M HCl in 50% MeOH	C_{18} symmetry (150 mm × 3.9 mm, 5 µm) reversed-phase column, coupled with C_{18} symmetry guard column (20 mmx3.9 mm, 5 µm) A: H_2O adjusted to pH 2.5 with TFA; B: Acetonitrile Flow rate: 1.0 mL/min column temperature, 40°C, UV–Vis photo diode array detection at 365 nm	[186]
16	Lingoberry, cranberry, onions, broccoli	Catechins, flavanones, flavones, flavonols	1.2M HCl in 50% MeOH	Inertsil ODS (150 mm × 4 mm; 3 µm) column coupled with C-18 guard A: 50 mM H_3PO_4 pH 2.5; B: acetonitrile Flow rate: 0.7 mL/min column temperature, 35°C, injection volume, 10 µL, UV–Vis photo diode array detection at 270, 329 amd 370 nm	[187]

(Continued)

Table 7.5 (*Cont.*)

Serial No.	Name of the plant	Category of polyphenols	Extraction solvent	LC condition used	Reference
17	Red raspberry	Phenolic acids, flavones	MeOH	Lichrocart 100 RP-18 (250 mm × 4 mm, 5 µm) A: 5% formic acid in H_2O; B: MeOH Flow rate: 1.0 mL/min Injection volume, 20 µL, UV–Vis photo diode array detection at 255 nm for phenolic acids and 360 for flavonoids	[188]
18	Apple ciders	Phenolic acids and flavonoids	Degassing, filtration	Nucleosil 120 C_{18} reversed phase column (250 mm × 4.6 mm, 3 µm) A: 2% CH_3COOH; B: MeOH; Flow rate: 0.8 mL/min column temperature, 25°C, injection volume, 50 µL, UV–Vis photo diode array detection at 313 nm (hydroxycinnamic acid derivatives), 355 nm (flavonol glycosides) and 280 nm (other phenolic compounds)	[189]

Serial No.	Name of the plant	Category of polyphenols	Extraction solvent	LC condition used	Reference
19	Artichoke	Phenolic acids and flavonoids	60%aqueous MeOH	Phenomenex C18 Hydro-Synergi column (150 mm × 3.0 mm, 4 µm) coupled with Phenomenex C_{18} ODS guard column (4 mm × 3 mm) A: 2% CH_3COOH; B: 0.5% CH_3COOH/acetonitrile (50:50); gradient	[190]
20	Grape skin	Anthocyanidins	water/ethyl alcohol (75/25)	C18SS Wakosil4.6 mm × 150 mm, 5 µm 0.1% TFA in water (A), 0.1% TFA in ACN (B) Flow rate: 1 mL/min, column temperature, 32°C, injection volume, 20 µL, UV–Vis photo diode array detection at 520 nm	[191]

(Continued)

Table 7.5 (*Cont.*)

Serial No.	Name of the plant	Category of polyphenols	Extraction solvent	LC condition used	Reference
21	Bayberry juice	Anthocyanins, flavonols and phenolic acids	Centrifuged (4000 rpm) and filtered (0.22μm)	C18Diamonsil 4.6 mm × 150 mm, 5 μm (for both anthocyanins, flavonols and phenolic acids) 0.1% Formic acid in water (A), 80% ACN in water (B) for anthocyanins 1% Formic acid (A), ACN (B) for flavonols Solvent A 4% acetic acid, and solvent B, methanol (A/B = 80/20, v/v) for phenolic acids Flow rate: 0.5 mL/min, Injection volume: 10 μL , UV–Vis photo diode array detection at 360 nm for flavonols Flow rate: 1.0 mL/min, Injection volume: 10 μL , UV–Vis photo diode array detection at 520 nm for anthocyanins Flow rate: 1.0 mL/min, Injection volume: 20 μL , UV–Vis photo diode array detection at 280 nm for phenolic acids	[192]

Serial No.	Name of the plant	Category of polyphenols	Extraction solvent	LC condition used	Reference
22	Red apricot	Phenolic acids	Acidified methanol	C18Phenomenex Gemini4.6 mm × 150 mm, 5 µm 0.1% Formic acid (A), 100% methanol (B) Flow rate: 0.7 mL/min, column temperature, 25°C, injection volume, 10 µL, UV–Vis photo diode array detection at 270. 325 nm	[193]
23	Onion	Flavonoids	70% v/v methanol/ water	C18Alltech Prevail2.1 × 150 mm3 µmC18 Hypersil2.1 × 150 mm 3 µm 0.1% Formic acid in water0.1% formic acid in water (A), 0.1% formic acid in methanol0.1% formic acid in acetonitrile (B) Flow rate: 0.2 mL/min, column temperature, 30°C, UV–Vis photo diode array detection at 280. 346, 364, 370 nm	[194]

Table 7.6 HPLC-UV conditions used for the determination of carotenoids.

SNo	Food material	Compound	Extraction solvents	LC conditions used	References
1.	Red and yellow tomato puree	lycopene	hexane / acetone / ethanol (50/25/25, v/v/v) Acetone/petroleum ether	HPLC,150×4.6mm C_{18} column, 1ml/min, 27°C, Acetonitrile (35–77%) in methanol	[167]
2.	Red and yellow tomato puree	β-carotene	a) hexane / acetone / ethanol (50/25/25, v/v/v) b) Acetone/petroleum ether	HPLC, 150×4.6mm C_{18} column, 1ml/min, 27° C, Acetonitrile (35–77%) in methanol	[167]
3.	Red bell peppers	Capsanthin (60% of the total carotenoids), *beta*-carotene (11%) and capsorubin (20%)		a) HPLC, ODS Resolve C_{18}, 1.8 ml/min, 470 nm, methanol-ethyl acetate	[202]

SNo	Food material	Compound	Extraction solvents	LC conditions used	References
4.	Paprika fruits and powder	Capsanthin, Capsorubin		HPLC, 10 µm Chromsil C_{18}, 1ml/min, 290 nm, 350 nm, 438 nm. a) acetone/ water (90/10, v/v) b) Acetone / water / hexane (85/10/5, v/v/v) c) acetonitrile/ 2 propanol/ water (39/57/ 6, v/v)	[203]
5.	Tomato	Lutein, lycopene, β-carotene, (their trans and cis- isomers)	Ethanol/hexane	UPLC, Phenomenex Kinetex 100×2.1 mm C_{18} 1.7 µm column, 300 µl/min, 30°C, 200–620nm monitored at 460nm. Methnol/MTBE/Water (solvent A, 85/14/5, v/v) and methnol/MTBE/water (solvent B, 90/5/5, v/v).	[204]

(Continued)

Table 7.6 (*Cont.*)

SNo	Food material	Compound	Extraction solvents	LC conditions used	References
6.	Lettuce, roquete, cress and chicory	neoxanthin, violaxanthin, lactucaxanthi-n, lutein and β-carotene.	Acetone and Petroleum ether	HPLC, C_{18} (Waters Spherisorb S3 ODS2), 3 mm, 4.6×150 mm. Acetonitrile, methanol, and ethyl acetate containing 0.05% of TEA (triethylamine). Flow rate of 0.5 ml/min.	[205]
7.	Yellow tamarillo fruits	Range of different carotenoids	ethanol/hexane, 4:3 v/v, containing 0.1% of BHT as antioxidant	C_{30} column (250 × 4.6 mm, 5 µm particle size), Mobile phase (water/20 mM ammonium acetate as eluent A, methanol/20 mM ammonium acetate as eluent B and MTBE as eluent C at 1 ml/min and the column, at 25°C). Injection volume was 20 µl. Absorbance at 290, 350, 400, 450, 470 nm.	[197]

SNo	Food material	Compound	Extraction solvents	LC conditions used	References
8.	Boiled broccoli, stir-fried broccoli, stir-fried endive, kale, green beans and stir-fried beans.	β-carotene, lutein, violaxanthin and neoxanthin	Acetone/petroleum ether and saponification when necessary	A C_{18} monomeric column (Spherisorb S3 ODS2), 3 mm, 4.6×150 mm, Mobile phase (acetonitrile/ methanol/ethyl acetate) at 0.5 ml/min.	[206]
9.	Coriander foliage	β-carotene	Acetone/petroleum ether in the presence of 0.1% BHT	C_{18} column (250×4.6 mm) isocratic mobile phase (acetonitrile: methanol: ethyl acetate in proportion of 80:10:10 v/v) at 1 ml/ min, 450 nm	[207]
10.	Tomato juice	Higher lycopene epoxide, lycopene, γ-carotene, β-carotene	THF stabilised with BHT (0.01%)	C_{18} Hypersil ODS column (250 × 4.6 mm i.d.; 5 µm particle size. Acetonitrile/ methanol/ dichloromethane/ hexane at 0.8 ml/min, 470 nm.	[208]

(Continued)

Table 7.6 (*Cont.*)

SNo	Food material	Compound	Extraction solvents	LC conditions used	References
11.	Orange juice	violaxanthin, lutein, zeaxanthin, β-cryptoxanthin, ζ-carotene, α-carotene, and β-carotene	Acetone	C_{18} (4.6 × 250 mm ID, 5 µm) column, acetonitrile/ methanol/ethyl acetate, at 0.7 ml/min, 450 nm.	[209]
12.	Pumpkin puree	Violaxanthin, lutein, ζ-carotene, α-carotene, trans-β-carotene, cis-β-carotene, Zeaxanthin, α-Cryptoxanthin,	acetone	C_{18} ODS2, 5 µm, 4.6 × 150 mm, acetonitrile (containing 0.05% of triethylamine)/ methanol/ ethyl acetate, at 0.5ml/min and 35°C.	[210]
13.	Taiwanese sweet potatoes	Cis and trans α- carotene and β-carotene	hexane/acetone/EtOH (2/1/1, v/v/v) containing 0.1 g of MgCO3 and 0.05 g BHT	C_{30} reverse-phase analytical column (250 × 4.6 mm, 5 µm) at 25 °C. MeOH/ACN/ H2O and CH_2Cl_2, at 1 ml/min, 450 nm.	[198]

SNo	Food material	Compound	Extraction solvents	LC conditions used	References
14.	Bendizao mandarin fruit peel	trans β-carotene	dichloromethane, ethanol, ethyl acetate, hexane, tetrahydrafuran	C_{30} column (250 × 4.6 mm diameter 5 µm) Methanol/ dichloromethane, 1.0 ml/ min, 35°C, 455 and 480 nm.	[199]
15.	Pumpkin	Lutein, lycopene, β-carotene, (their trans and cis- isomers), α- carotene	$SCCO_2$ or Hexane	C_{30} column (250 mm × 4.6 mm i.d., 5 µm particle size). methanol/ methyl tert-butyl ether (MTBE)/water (81:15:4, v/v/v; A), and methanol/ MTBE/water (4:92:4, v/v/v; B). at 450 nm, 0.42 mL/min	[200]
16.	Carrot, spinach, tomatoes, corn and tangerines	lutein, zeaxanthin, /3-cryptoxanthin, α- carotene, and cis and trans of β-carotene and lycopene	Methanol/THF (1:1 v/v)	C_{18} reverse phase column 250 × 4.6 mm, 5 µm particle size. Methanol and THF (95:5 v/v), 1ml/min, 450 nm.	[212]

(Continued)

Table 7.6 (*Cont.*)

SNo	Food material	Compound	Extraction solvents	LC conditions used	References
17.	canned tomato juice, carrot and spinach	α- carotene, carotene, lycopene and β-apo-8´-carotenal	petroleum ether: isopropanol (1:3) or diethyl ether dichloroethane: methanol (1:1) or hexane, diethyl ether acetone, petroleum ether acetone: hexane (2:3) or acetone, hexane or ethanol: hexane (4:3), ethanol, hexane or tetrahydrofuran (for high carotene content) or tetrahydrofuran, petroleum ether (for low carotene content) or tetrahydrofuran acetone:petroleum ether (1:1) acetone, or methanol:acetone (1:1) or acetone, diethyl ether	C_{18} reverse phase column (150×13.9 mm, particle size 5 µm)	[213]

SNo	Food material	Compound	Extraction solvents	LC conditions used	References
18.	carrots puree, and juices	Lutein, retinol, β-carotene, α- carotene	BHT in ethanol,	C_{30} column,30 °C, methanol, isopropanol with or without methyl tert-butyl ether, 325nm, 450nm and 1ml/min	[201]
19.	Tomato, carrot	xanthophyll, lycopene, α and β-carotene	Methanol and hexane	C_{18}, 25cm ×4.6mm × 5µm column, methanol-acetonitrile-chloroform (47:47:6) or acetonitrile-dichloromethane, 2ml/min and 461nm	[214]
20.	Pitanga fruit	Lycopene, rubixanthin and β-cryptoxanthin	$SCCO_2$	C_{18}, 300mm×3.9mm × 4µm column, acetonitrile/H_2O/ethyl acetate, 1mL/min, at 29 C and 450nm	[211]
21.	Spinach	Lutein, β-carotene, violaxanthin and neoxanthin	methanol/ethyl acetate/petroleum ether (1:1:1, v/v/v)	C_{18}, 250mm×4.6 mm×5 µm column, acetonitrile: water (9:1, v/v), with 0.25% triethylamine (solvent A) and ethyl acetate with 0.25% triethylamine (solvent B). 1ml/min, 450nm.	[170]

C_{30}-bonded silica [197–201] exhibits a higher selectivity than the conventionally used C_{18} material [170, 202–211] (Table 7.6). The separation behaviour of carotenoids on C_{30} silica phases is strongly temperature-dependent, with best separations obtained at lower temperatures [197). The addition of antioxidants such as butylated hydroxytoluene (BHT) to the mobile phase (and to the extraction solvent) has been reported to avoid oxidisation [201, 208]. Carotenoids are coloured compounds due to their conjugated double bonds, where the maxima in their absorption spectra, spanning from 380 to 550 nm, shifts to longer wavelengths with increasing number of conjugated double bonds.

7.6.3 Liquid Chromatography–Mass Spectrometry (LC–MS)

Nowadays, the liquid chromatography–mass spectrometry (LC–MS) technique is the best analytical approach to study phytochemicals from different biological sources, and is the most effective tool in the study of their structure [215, 216], particularly tandem mass spectrometry. However, mass spectrometry alone can not provide complete structural information of the compound especially when isomers of bioactive compounds are studied [217]. Mass spectrometry in combination with NMR techniques is the most popular methodology for conferring a structure to an unknown compound. In principle, the mass spectrometry ionises chemical compounds to generate charged molecules or molecule fragments and measures their mass-to-charge ratios [218]. The main sources used to ionise phytochemicals are: fast atom bombardment (FAB), electrospray ionisation (ESI), atmospheric pressure ionisation (API) and matrix-assisted laser desorption ionisation (MALDI).

7.6.3.1 Phenols

Electrospray ionisation has been used to establish polyphenol fingerprints of complex herb (Lamiaceae) extracts [219]. The matrix-assisted-laser-desorption-ionisation-time-of-flight (MALDI–TOF) technique is suitable to determine the presence of molecules of higher molecular weight with high accuracy, and it has been applied with success to study procyanidin oligomers [220]. The structural heterogeneity of the polyphenols from cranberries, grape seed extracts, sorghum and pomegranate was characterised by MALDI–TOF MS. The spectrometric analysis gave information on the degree of polymerisation, monomeric substitution, and the nature of intermolecular bonds [221]. Ionisation of target molecules is very important for the MS analysis of polyphenols.

Most studies on polyphenols, with the exception of anthocyanins, used negative ionisation mode (Table 7.7). Anthocyanins are best resolved in positive ionisation mode, whereas phenolic acids and flavonoids show higher sensitivity in negative mode [219]. Most mobile phases used in LC-MS studies consist of weak organic acids such as formic acid, trifluoroacetic acid and acetic acid. The presence of acid in the mobile phase is essential for the complete separation of certain polyphenols, for example, catechins and the sharp peak shape [222].

7.6.3.2 Carotenoids

In complex matrixes, when diode array detector is insufficient for identification because of spectral interferences, mass spectrometry coupled with liquid chromatography has been successfully used in the case of carotenoids (Table 7.8). The LC-MS method developed for carotenoid analysis mainly includes atmospheric pressure ionisation interfaces (APCI) [243, 244] or electrospray ionisation interfaces (ESI) [245, 246].

Lacker *et al.* [243] developed a method for the identification of carotenoid mixture, including astaxanthin, cantaxanthin, zeaxanthin, echinenone and β-carotene, as well as cis–trans isomers of β-carotene using LC-MS in the APCI mode. Mass spectra were acquired over the 300–2000 m/z scan range. The detection limits obtained in positive-ion mode were in the nanogram range. Several authors have used mass spectrometry to ensure correct peak identification and purity in a complex matrix. Gentili and Caretti [247], Gayosso-García Sancho *et al.* [248], Azevedo-Meleiro and Rodriguez-Amaya [244] and Lacker *et al.* [243] used LC-MS-APCI in positive-ion mode to confirm the carotenoids present in different fruits and vegetables. An HPLC-MS (ESI) was used by Thompson and Loa [250] to confirm the carotenoids present in 'Bhut Jolokia' pepper extract.

7.6.4 Liquid Chromatography–Nuclear Magnetic Resonance (LC–NMR)

NMR spectroscopy is the emerging technology to analyse foods and their bioactive compounds. Despite high equipment cost, this technology is widely used in the field of natural product chemistry due to its advantages such as convenience in sample preparation, simplicity of operating procedures and the instrumental stability. NMR is probably the best non-target technique to use for the screening of food extracts: all the main metabolites (fatty, amino and organic

Table 7.7 Studies of polyphenols using LC-MS.

Serial No.	Name of the plant	Category of polyphenols	Extraction condition	LC-MS condition used	Reference
1	Almond	Flavonoids and phenolic acids	3% acetic acid, 50% methanol in water	Column: 250 x 4.60 mm Synergi 4µm MAX-RP 80A Mobile phase: An increasing gradient of 1% formic acid (A) and 100% methanol (B) Flow rate: 0.2 mL/min Coulmn temperature: 25 °C Ionisation mode: Negative mode used	[223]
2	*Salvia officinalis*	Flavonoids and phenolic acids	boiling water	Column: Waters Acquity BEH Shield RP18 column (1.7 µm x 150 mm x 2.1 mm) Mobile phase: 0.1 % Formic acid in deionised water (A) and 100% acetonitrilel (B) Flow rate: 0.4 mL/min Ionisation mode: Negative mode used	[224]

Serial No.	Name of the plant	Category of polyphenols	Extraction condition	LC-MS condition used	Reference
3	Kiwi fruit *(Actinidia deliciosa)*	Mostly phenolic acids	ethanol-water (50:50 v/v)	Column: Macherey–NagelNucleoder C18 Gravity column (5 µm x 125 mm x 2 mm) Mobile phase: Isocratic elution with 100% methanol containing 5% formic acid Flow rate: 0.3 mL/min Ionisation mode: Negative Coulmn temperature: 30 °C Injection volume: 5 µL	[225]
4	Carob fibre	Flavonoids and phenolic acids	distilled water	Column: Reversed phase C-18 column (5 µm x 250mm × 4 mm) Mobile phase: An increasing gradient of 2% acetic acid (A) and 100% methanol (B) Flow rate: 0.5 mL/min Ionisation mode: Negative Injection volume: 5 µL	[226]

(Continued)

Table 7.7 (*Cont.*)

Serial No.	Name of the plant	Category of polyphenols	Extraction condition	LC-MS condition used	Reference
5	Curry leaf	Flavonoids	80% acetone, 100% methanol, 80% methanol, or 80% ethanol in 20% water.	Column: Pursuit 3 C18 column (3 µm x 150 mm × 3 mm) Mobile phase: Solvent A: 10% methanol in water (pH 3.5 with 0.01% formic acid) and Solvent B: 20% methanol, 20% water (pH 3.5 with 0.01% formic acid), and 60% acetonitrile Flow rate: 1.0 mL/min Ionisation mode: Negative Injection volume: 1.5 µL	[227]
6	Lamiaceae herbs	Flavonoids and phenolic acids	80:20 (v/v) methanol–water solution	Column: Atlantis T3 C18 column (Waters Corp., Milford, MA; 100 mmx2.1mm; 3 µm particle size) Mobile phase: 0.5% aqueous formic acid (solvent A) and 0.5% formic acid in 50:50 v/v acetonitrile:methanol (solvent B). Flow rate: 0.2 mL/min Ionisation mode: Negative Injection volume: 3 µL	[219]

Serial No.	Name of the plant	Category of polyphenols	Extraction condition	LC-MS condition used	Reference
7	Muscadine grapes (*Vitis rotundifolia*)	Anthocyanins	80:20 (v/v) methanol–water solution containing 0.1 mL/L HCl	Column: Symmetry C18 (5 µm x 150 mm × 3 mm) (Waters Corporation, Milford, MA) Mobile phase: 5:95 (v/v) formic acid–water solution (phase A) and 5:95 (v/v) formic acid–methanol solution (phase B) Flow rate: 0.8 mL/min Ionisation mode: Positive Injection volume: 1.5 µL	[228]
8	Fig fruit (*Ficus carica* L.)	Anthocyanins	MeOH containing 0.5% trifluoracetic acid (TFA)	Column: AQUAs (Phenomenex, Torrance, CA) reversephaseC18 column (5 µm, 150mm_4.6mm i.d.) thermostatted at 35 °C. The solvents used were: (A) 0.1% TFA in water and (B) 100% HPLC-grade acetonitrile Flow rate: 0.5 mL/min Ionisation mode: Positive	[229]

(Continued)

Table 7.7 (*Cont.*)

Serial No.	Name of the plant	Category of polyphenols	Extraction condition	LC-MS condition used	Reference
9	Purple corn (*Zea mays* L.)	Anthocyanins	1.5M HCl–95% ethanol (15:85)	Column: Lichrospher C-18 (5 µm 2.1 mm×250 mm i.d.) Mobile phase: Phase A was a mixture of 0.05% (v/v) trifluoroacetic acid (TFA) in distilled water, whereas phase B consisted of 100% HPLCgrade acetonitrile. Flow rate: 0.8 mL/min Ionisation mode: Positive Injection volume: 10 µL	[230]
10	Strawberry (Camarosa variety) *Fragaria ananassa*	Anthocyanins	Aqueous puree extracted with solid-phase extraction method. SPE eluent: methanol/acetic acid, 19:1	Column: (5 µm x 250mm × 4.6 mm) C18 Luna column (Phenomenex, Germany) Mobile phase: solvent A (water/acetonitrile/formic acid, 87:3:10, v/v/v) and solvent B (water/acetonitrile/formic acid, 40:50:10, v/v/v) Flow rate: 0.5 mL/min Ionisation mode: Positive Injection volume: 50 µL	[231]

Serial No.	Name of the plant	Category of polyphenols	Extraction condition	LC-MS condition used	Reference
11	Pomegranate	Flavonoids, phenolic acids and anthocyanins	For anthocyanins: aqueous methanol (80%, v/v; 0.1% HCl) For non-anthocyanins: ethylacetate	Phenomenex (Torrance, CA, USA) C 18 Synergi (4 µm x 150mm × 3 mm) a. Anthocyanin 5% (v/v) formic acid in water (eluent A) and of water, formic acid and methanol (10/10/80, v/v/v; eluent B). Flow rate: 0.4 mL/min Ionisation mode: Positive Injection volume: 10 µL b. non-anthocyanin 2% (v/v) acetic acid in water (eluent A) and of 0.5% acetic acid in water and methanol (10/90, v/v; eluent B Flow rate: 0.4 mL/min Ionisation mode: Positive Injection volume: 15 µL	[232]

(Continued)

Table 7.7 (*Cont.*)

Serial No.	Name of the plant	Category of polyphenols	Extraction condition	LC-MS condition used	Reference
12	Tomato and tomato products	Flavonoids and phenolic acids	80% ethanol in Milli-Q water (4 ml);	Luna C18 column 50 _ 2.0 mm internal diameter, 5 lm (Phenomenex, Torrance, CA, USA) Mobile phases consisted of 0.1% formic acid in Milli-Q-water (A) and 0.1% formic acid in acetonitrile (B). Flow rate: 0.4 mL/min Ionisation mode: Negative	[233]
13	Tea	Flavonoids	Acetonitrile–water, 1:1, v/v	Gemini C18 reverse phase column (250mm x 4.6mm i.d., 5 µm, Phenomenex, Torrance, CA, USA), protected with a security guard cartridge (Gemini C18, 4_2.0mm i.d., Phenomenex). The elution consisted of a linear gradient program from 4% to 25% acetonitrile in 1% formic acid aqueous solution HPLC UV-Vis: 240–245 nm and around 270 nm Flow rate: 1.0 mL/min Ionisation mode: Negative Injection volume: 10 µL	[234]

Serial No.	Name of the plant	Category of polyphenols	Extraction condition	LC-MS condition used	Reference
14	lettuce (*Lactuca sativa* L.)	Flavonoids and phenolic acids	MeOH/water/formic acid (25/24/3) (v:v:v)	LiChroChart C18 column (250mm x 4 mm; 5 µm particle size; The mobile phases were water with 5% formic acid (A) and methanol (B) with a in a gradient program starting with 5% B in A, reaching 40% B at 25 min, and then remaining isocratic for 5 min. The UV chromatograms were recorded at 330 and 520 nm. Flow rate: 1.0 mL/min Ionisation mode: Negative Injection volume: 10 µL	[235]

(Continued)

Table 7.7 (*Cont.*)

Serial No.	Name of the plant	Category of polyphenols	Extraction condition	LC-MS condition used	Reference
15	Citrus fruits	Flavonoids and phenolic acids	Ethyl acetate	Discovery C18 (250 mm x 4.6 mm id, 5 µm) column (Supelco, Pennsylvania, USA) The solvents used were: 0.1% acetic acid in water (solvent A) and acetonitrile (solvent B) with the following linear gradient. The column temperature was 25°C The diode array detector was set at 280 nm for the quantitative determination of flavanones, at 335 nm for flavones and flavonols Flow rate: 0.6 mL/min Ionisation mode: Negative Injection volume: 10 µL	[236]
16	Paprika	Flavonoids	Methanol (MeOH), ethanol (EtOH), N-N-dimethylformamide (DMF), DMF:EtOH (50:50, v/v), DMF:MeOH (50:50, v/v), and EtOH:water (80:20, v/v)	C18 Phenomenex column (Torrance, CA, USA) Gemini series (250mm x 4.6 mm i.d.,5 µm particle size) Solvent A (0.03 M phosphoric acid in water) and B (MeOH), and the flavonoids were detected at 360 nm. Flow rate: 1.0 mL/min Ionisation mode: both the negative or positive ion mode. Injection volume: 30 µL (in HPLC), 7 µL (in MS)	[237]

Serial No.	Name of the plant	Category of polyphenols	Extraction condition	LC-MS condition used	Reference
17	Cucumber	Phenolic acids and flavonoids	80:20 (v/v) methanol/ H_2O	Zorbax C18 column (4.6 × 150 mm, 1.8 µm) Acidified water (0.5% acetic acid, v/v) and acetonitrile were used as mobile phases A and B respectively The flow rate was set at 0.80 mL/min throughout the gradient The column temperature was maintained at 25°C and the injection volume was 10 µL. using negative ion mode with spectra acquired over a mass range from m/z 50 to 1100. The optimum values of the ESI-MS parameters were: capillary voltage, + 4.0 kV; drying gas temperature, 190°C; drying gas flow, 9.0 L/min;	[238]

(Continued)

Table 7.7 (*Cont.*)

Serial No.	Name of the plant	Category of polyphenols	Extraction condition	LC-MS condition used	Reference
18	chickpea, red kidney bean, haricot bean, yellow lentil, red lentil and green lentil)	Isoflavones	10 mL of methanol (MeOH) and 2 mL of 0.1 N HCl	50 mm × 2.1 mm ID 3 µm Zorbax Eclipse XDB C18 column at 35°C. 0.01% (w/v) formic acid and 5 mM ammonium formate in water as mobile phase A and acetonitrile as the mobile phase B The flow rate was 0.3 mL/min. The injection volume was 5 µL. The electrospray ionisation (ESI positive + negative) source was used in the MRM mode at a capillary voltage of 3500 V. The drying gas flow was set at 10.0 L/min at 350°C. MS and MS/MS spectra were collected in full- scan mode over a range of m/z 50–500	[239]

Serial No.	Name of the plant	Category of polyphenols	Extraction condition	LC-MS condition used	Reference
19	Eggplant	Phenolic acid, flavonoids and their derivatives	Aqueous methanol (80%)	Hypersil Gold C_{18} (3 µm particle size; 150 mm length × 3.0 mm) Solvent A consisted of 10% methanol in H_2O adjusted to pH 3.5 with formic acid. Solvent B consisted of 20% H_2O (pH 3.5 with formic acid), 20% MeOH, and 60% acetonitrile. At a flow rate of 0.3 ml/min Mass spectra were acquired in the negative ion mode under the following parameters: capillary voltage, 3 kV; source block temperature, 120°C; desolvation gas temperature, 400°C. Nitrogen was used as the drying and nebulizing gas at flow rates of approximately 50 and 450 l/h. For full-scan ◄HPLC►–ESI–MS analysis, spectra were scanned in the range of 50 to 1200 *m/z*.	[240]

(Continued)

Table 7.7 (*Cont.*)

Serial No.	Name of the plant	Category of polyphenols	Extraction condition	LC-MS condition used	Reference
20	Blue berries	Flavonol glycosides	60 g of fresh fruit was homogenised in a model 847–86 Osterizer blender in 250 mL of mixture acetone/water (70/30, v/v) for 1 min	Aqua 3u, C18, 125 A, 150 × 2 mm, A (2% acetic acid in water) and B (0.5% acetic acid in acetonitrile/water 50:50). flow rate was 0.3 mL/min and oven temperature at 40°C. The injection volume was 10 µL capillary voltage 3000 V, extractor voltage 5 V, source temperature 110°C, desolvation temperature 250°C, cone gas flow (N_2) 50 L/h, desolvation gas flow (N_2) 400 L/h mass spectra ranging from *m/z* 100 to 800 were taken in positive mode UPLC performed in positive mode under the following conditions: capillary voltage 3 kV, sampling cone 25 V, extraction cone 3 V, source temperature 150°C, desolvation temperature 500°C, cone gas flow (N_2) 50 L/h, desolvation gas flow (N_2) 1000 L/h. The *m/z* range was 50–1000 Da	[241]

Serial No.	Name of the plant	Category of polyphenols	Extraction condition	LC-MS condition used	Reference
21	Potato tubers	Phenolic acids	water/methanol (50/50, v/v) containing 0.5% (v/v) acetic acid	Hypersyl gold RP column 150 mm × 2.1 mm i.d.; particle size 1.9 µm) at 30°C. The eluents used were water/acetonitrile/acetic acid (99:1:0.1, v/v/v) (A) and acetronitrile/acetic acid (100:0.1, v/v) (B). The flow rate was 400 µL/min; injection volume was 5 µL. Detection in the negative ion mode, with a source voltage of 3.5 kV, and an ion transfer tube temperature of 350°C. A full-scan mass spectrum over a range of m/z values of 150–1500 was recorded	**[242]**

Table 7.8 LC-MS conditions used for the determination of carotenoids

SNo	Food material	Compound	Extraction solvents	LC-MS conditions	References
1.	Tomatoes, carrots, spinach and celery	echinenone, canthaxanthin, astaxanthin, zeaxanthin, β-carotene	MTBE	HPLC–UV–APCI–MS, C_{30}, 250 ×4.6 mm column packed with ProntoSil silica gel (3 µm, 200 Å) modified with triacontyltrichlorosilane, m/z 200–700, capillary temperature 150°C in positive ion mode.	[243]
2.	'Bhut Jolokia' pepper		Methnol:Water	LC-ESI-MS, C_{30} column (4.6 × 150 mm; 3 µm particles; 30°C), isocratic mobile phase, either 37% water: 63% methanol, flowing at 0.75 ml/min or 30:70 aqueous methanol at 0.80 ml/min, at m/z 137 and m/z 275–350, p[ositive ion mode.	[250]
3.	Apricots, Pumpkins	trans-β-carotene, zeaxanthin, antheraxanthin, γ-carotene, lycopeneand lutein	acetone and hexane (1:1, v/v) containing butylated hydroxytoluene (50 mg/ 100 mL) and butylated hydroxyanisole (50 mg/100 mL) as antioxidants.	HPLC–UV–APCI–MS, 450 nm at 0.42 mL/min, 150 × 3.0 mm i.d., 3 µm particle size, C_{30} column operated at 25°C. methanol/ methyl tert-butyl ether (MTBE)/ water (81:15:4 v/v/v; eluent A) and methanol/ MTBE/ water (4:92:4, v/v/v; eluent B). m/z 100–1100, capillary voltage 2779 kV at a temperature of 350°C in positive ion mode.	[244]

SNo	Food material	Compound	Extraction solvents	LC-MS conditions	References
4.	Acerola, pitanga,. Pequi, and camu-camu	Neoxanthin, Violaxanthin, Lutein, Zeaxanthin, Rubixanthin, β-cryptoxanthin, β-carotene-5,6-epoxide Lycopene, Cis-lycopene, γ-carotene α-carotene β-carotene	Acetone	HPLC-MS, Electron impact mass detector, C_{18}, 3 µm, 4.6 i.d.×150 mm, mobile phase acetonitrile (containing 0.05% triethylamine), methanol and ethyl acetate, column nebulizer temperatures being 80°C and 90°C, ionizing voltage 70 eV and the temperature of the ion source 210°C. The m/z 150–650.	[249]
5.	Papaya fruit	β-Cryptoxanthin, β-Carotene, Lycopene	Hexane/ Dichloromethane	HPLC-APCI-MS-TOF, drying gas temperature 350°C, corona, capillary, fragmentor, and skimmer voltages were 4 µA, 4 kV, 200 V, and 60 V, respectively. mass spectra (m/z 100–800	[248]

(Continued)

Table 7.8 (*Cont.*)

SNo	Food material	Compound	Extraction solvents	LC-MS conditions	References
6.	Maize flour, green and golden kiwi	lutein, zeaxanthin, β-cryptoxanthin, and β-carotene and α-tocopherol, δ-tocopherol, γ-tocopherol, ergocalcif-erol, phyllo-quinone and menaquinone-4),	isopropanol:hexane (50:50, v/v) solution	HPLC-APCI-MS, C30 column (250×4.6mm i.d., particle size 3 μm) protected by a guard column of the same type (4.0×10mm, 5μm particle size), mobile flow rate 1ml/min. Needle current of 3μA and a probe temperature of 450 C. High-purity nitrogen used as curtain (2 L/min) and collision (4 mTorr) gas, while air as nebulizer (1.5 Lmin−1) and make-up (2 Lmin−1) gas. 1ml/min	[247]

acids, sugars, polyphenolic, carotenoid compounds) can be detected in a single spectrum with minimal and non-destructive sample preparation [256]. Standard 1H, 13C and high-resolution magic angle spinning (HR/MAS) NMR spectra carry a lot of chemical information used to compare, discriminate or classify the samples. Selected variables (NMR peak heights or integrals) that characterise the samples in a specific way are also used instead of the whole spectra. Chemometric techniques are often employed to analyse the data as the information contained in the spectra is highly complex. The preparation of the food sample is actually simple for non-hyphenated NMR experiments, depending on the nature of the sample (liquid, solid). In some cases, previous extraction or fractionation step is required while other samples may be used without preparation. For high resolution 1H, 13C, 31P NMR of aqueous liquids (fruit juices, degassed beer, wine, etc.) the samples are often prepared simply by adding 5–10% of D2O to the liquid [258]. Deuterated solvents provide a signal for magnetic field stabilisation and allow optimisation of the NMR peak resolution. Solid samples (fruits, vegetables, green tea) are freeze-dried, ground and then extracted in a deuterated solvent. Other samples, such as oils or instant coffees are simply dissolved at the desired concentration in a suitable deuterated solvent. The main limiting factor of NMR other than cost is its relatively low sensitivity compared to other techniques such as HPLC-MS or GC-MS. Low sensitivity particularly hinders the more sophisticated 2D NMR experiments such as COSY, NOESY and HMQC. Therefore, purification and concentration of the analytes are often required to get clear spectral data which is necessary to achieve detailed structure of the analytes. Introduction of LC and solid-phase extraction (SPE) before NMR analysis perform the job of purification and concentration. For this reason, numerous studies have used LC-NMR or LC-SPE-NMR to analyse polyphenols in plant extracts (Table 7.9). Exarchou *et al.* [259] analysed oregano plant extracts with very promising results using LC-SPE-NMR technique. In this case, a SPE unit was inserted between the LC–DAD and NMR spectrometer, in order to trap the eluting compounds onto SPE cartridges. Each one of the trapped compounds was eluted into the NMR probe with deuterated solvent and analysed using various NMR experiments. A combination of LC-MS and LC-SPE-NMR was used for assigning structure to the polyphenols of St. John's wort (*Hypericum perforatum*). Application of LC-MS simultaneously with LC-SPE-NMR provided useful information which supported the structural elucidation of the NMR experiments obtained by ^{1}H NMR, ^{1}H–^{1}H COSY and ^{1}H–^{1}H TOCSY.

Table 7.9. Selected LC-NMR studies of plant polyphenols

Serial No.	Name of the sample	Category of polyphenols	NMR type	Solvent used	LC condition used	Reference
1	Metabolites of Black tea	Gallic acid derivatives	Bruker DRX 600	D_2O	250 x 4.6 mm i.d., 5 µm, Chromspher PI C18 reversed phase column Mobile phase: isocratically using 96:4 v/v deuterium oxide/ acetonitrile with 0.4% formic acid (v/v) Volume flow cell: 120 µL Injection volume: 100 µL	[251]
2	*Gentiana ottonis*	Flavonoids	Unity Innova 500 MHz	MeOH, D_2O	Nova-Pak C column, 4 mm (150x3.9 mm I.D.) from Waters (Bedford), equipped with a Nova-Pak Guard-Pak C18 Mobile phase: D_2O, MeCN, TFA Volume flow cell: 60 µL Injection volume: 10–20 µL	[252]

Serial No.	Name of the sample	Category of polyphenols	NMR type	Solvent used	LC condition used	Reference
3	*Hypericum perforatum*	Flavonoids	Bruker DPX-400 NMR	deuterated acetonitrile	150 mm · 4.6 mm i.d. Altima C18 column from Alltech (Breda, The Netherlands) Mobile phase: solvent A (0.1% v/v formic acid in water) and solvent B (0.1% v/v formic acid in acetonitrile) Volume flow cell: 120 µL Injection volume: 25 µL	[253]
4	Apple peel	Flavonoids	DRX-500	D_2O, CD_3OD	Mobile phase: D_2O, MeCN, TFA Volume flow cell: 120 µL Injection volume: 100 µL	[254]

(Continued)

Table 7.9 (*Cont.*)

Serial No.	Name of the sample	Category of polyphenols	NMR type	Solvent used	LC condition used	Reference
5	Leaves *Sorocea bomplandii*	Flavonoids	AMX 600	MeOH	250x4.6 mm stainless steel column (Bischoff, Leonberg, Germany) with a particle size of 3 µm Mobile phase: D_2O, MeOH Volume flow cell: 120 µL Injection volume: 10 µL	[255]
6	*Lycopersicon esculentum*	Flavonoids	DMX 500	CD_3OD, D_2O	Column: JAFC Mobile phase: D_2O, MeCN, TFA Volume flow cell: 120 µL Injection volume: 60–100 µL	[258]
7	Grape berry skin	Anthocyanins	BRUKER AVANCE III 600 MHz	H_2O, CH_3CN	Prontosil C18 (5µm, 250 mm × 4.6 mm), Bischoff (Leonberg, Germany) Mobile phase: deuterated water (solvent A), aceto-nitrile–0.25% TFA (solvent B) and water–0.25% TFA (solvent C) Volume flow cell: 120 µL Injection volume: 10 µL	[257]

7.7 Concluding Remarks

In spite of certain discrepancies related to the 'antioxidant hypothesis', the message that antioxidants are good for health has considerable momentum worldwide. Today, nutrition and other scientific societies tend to recognize the importance of dietary antioxidants as agents for promoting health and wellbeing. The key nutritional role of foods of plant origin is unquestionable, due to the massive presence of phytochemicals with various biological activities. These plant foods are good sources of major and minor compounds which may have important metabolic and/or physiological effects. More recent evidence provides potential information of their impact on health, so these secondary metabolites are currently marketed as functional foods and nutraceutical ingredients. The authors would like to highlight the fact that there are many methods used to determine total antioxidant activity, and it is important to point out that all of them have some limitations. It has been observed in previous studies that some antioxidant assay methods give different trends. For that reason multiple methods to generate an 'antioxidant profile' might be needed.

The most common method to analyse these antioxidants is reversed-phase HPLC with UV detection. This UV detection can be a simple UV-Vis detector or DAD. Various solvents can be used, but are usually selected from an organic phase, ACN or methanol and an aqueous phase, water acidified or not. Identification and quantitation, based mainly on HPLC and LC–MS methods can be aided today by NMR methodology, especially homonuclear two-dimensional correlated spectroscopy, $H^{1-13}C$ heteronuclear multiple-bond correlation spectroscopy and other techniques such as LC-NMR or LC-NMR/MS, which may provide information on the overall composition and enable the identification of individual phenols and caroteniods in complex matrices.

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8

Indispensable Tools in Food Science and Nutrition

Sneha P. Bhatia

Essex County College, Newark, New Jersey

Abstract

The modern consumer craves a wide variety of foods from different parts of the world. Ensuring that these food products retain their nutritional content and flavor through this process has become an increasingly important factor. A strong regulatory framework along with the discipline of Microbiology and Genetics serve as overarching pillars and are indispensable tools that advance the science of food safety and nutrition. This chapter will provide an overview on foodborne pathogens, probiotics and genetically modified foods.

Keywords: Food safety, genetically modified (GM) foods, food science, nutrition

8.1 Introduction: Food Safety – From Farm to the Dinner Plate

We live in a global society. The modern consumer demands a wide variety of foods from different parts of the world. This has significantly impacted the import and export of a variety of food products. Moreover, dinners that would usually take hours to prepare, can now be prepared in a few minutes. Economically, this increasing demand has created new opportunities. At the same time, this also exposes these food products to transportation contamination, adulteration and spoilage. Moreover, climate change has become as

**Corresponding author*: bhatia.sneha@gmail.com

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increasing factor that is impacting foodborne pathogens. Ensuring that these food products retain their nutrition content and flavor through this process has become an increasingly important factor. Food science is an interdisciplinary science that uses chemical engineering, molecular biology, microbiology, environmental science, botany, statistics and informatics to study all the steps involved from food production to consumption; which includes harvesting, processing, flavoring, packaging, storing conditions to consumption of food; essentially from farm to the dinner table.

Case Scenario – Impact of Climate Change on Outbreak of *Vibrio parahaemolyticus*

In 2004, an outbreak of diarrhea was reported on a cruise ship that was sailing on Prince William Sound waters. Extensive analysis of the stool samples of the patients revealed presence of *Vibrio parahaemolyticu*s. *V. parahaemolyticus* is a gram negative halophilic (salt loving) bacteria that inhabits warm estuarine waters. It requires a minimum water temperature of 16.5°C to survive and the waters of Prince William Sound have historically been colder than that. Before the summer of 2004, Alaskan waters were thought to be too cold to support levels of *V. parahaemolyticus* high enough to cause disease. However, the rising temperatures of ocean water seem to have contributed to this outbreak of *V. parahaemolyticus* [7, 33].

Just within the United States, over 47 million cases of foodborne illness occur every year. Food safety is an interdisciplinary science that focuses on preventing foodborne illness. Foodborne illness (also commonly referred to as food poisoning) is any illness that occurs from eating contaminated food. Contaminated food can be defined as food that contains hazards or contaminants. These hazards might be naturally present or can be accidently or deliberately introduced. These hazards can be classified as:

- Biological – microbial pathogens such as bacteria, viruses, prions, parasites such as fungi and poisonous plants.
- Chemical – pesticides, heavy metals, and cleaning materials. (e.g. Melamine was deliberately added at milk stations in China to dilute raw milk and increase the protein levels. Over 300,000 infants and children

were affected with kidney stones and urinary tract effects. This was one of the largest deliberate food contamination incidents.)
- Physical hazards – metals, staples, glass, materials that are accidently or deliberately introduced.

Although, some food cases of foodborne illnesses can be linked to either natural toxins or chemical toxins. Generally, a majority of the food poisoning cases are related to bacteria, viruses and other microbial pathogens, as opposed to toxic substances in the food. Many of the bacteria responsible for foodborne diseases also exist in healthy animals. This makes it impossible to detect these agents by visual examination [8].

8.2 Foodborne Pathogens

Microbes are almost everywhere. They are found in the soil, air, and intestines of animals and humans. Hence, microbes are also found in food. Most of the time they may not cause harm and can even be beneficial, but certain strains cause harmful effects in living organisms. Foodborne pathogens are microbes that are found in food and result in food spoilage. Often times, food spoilage can be detected by foul odor or change in the physical characteristics of the food. However, often times foodborne pathogens are not identified by these means.

Foodborne pathogens can be bacteria, viruses, prions, protozoa, fungi or poisonous plants. Most common types of pathogens that cause foodborne illness as surveyed by the Centers for Disease Control (CDC) are reported in Tables 8.1 and 8.2. The common microbial pathogens in the United States are: *Salmonella,* Norovirus, *Clostridium perfringens, Campylobacter spp, Listeria monocytogenes,* and *E.coli.* It is important to note that only some strains of these microbes will cause foodborne illness [7, 10].

Salmonella is a gram negative rod-shaped (bacillus) motile bacterium. It is generally found in contaminated food, water and infected animals. Nontyphodial strains cause diarrhea, fever, and abdominal cramps. Generally people infected with diarrhea recover rapidly. However, often times Salmonella can spread from the intestines to the blood stream and cause localized infection and even lead to

Table 8.1 Top five pathogens contributing to domestically acquired foodborne illnesses resulting in hospitalization [7].

Pathogen	Estimated number of hospitalizations
***Salmonella*, nontyphoidal**	19,336
Norovirus	14,663
Campylobacter spp.	8,463
Toxoplasma gondii	4,428
***E.coli* (STEC) O157**	2,138

Table 8.2 Top five pathogens contributing to domestically acquired foodborne illnesses resulting in death [7].

Pathogen	Estimated number of deaths
***Salmonella*, nontyphoidal**	378
Toxoplasma gondii	327
Listeria monocytogenes	255
Norovirus	149
Campylobacter spp.	76

death, unless it is treated with appropriate antibiotic. Salmonella can also progress into Reiter's syndrome, which is characterized by irritation of the eyes, painful urination, and joint pain. This can last for several months. Patients with Reiter's syndrome often develop arthritis. *Newport, typhimurium,* and *Enteritidis* are the most common Salmonella serotypes that result in foodborne illness. Eggs, raw milk, animal products, chicken, and uncooked meat are known to cause Samonellasis [7, 10].

Noroviruses are a group of naked ssRNA viruses. The most common symptoms of acute gastroenteritis are diarrhea, vomiting, and stomach pain. Noroviruses spread very rapidly. They are easily spread through contaminated water, food, contaminated surfaces, or are even spread during the harvesting stage (e.g., oysters harvested from contaminated waters). Infection with noroviruses is commonly referred to as "stomach flu." Gastroenteritis is commonly observed in patients infected with norovirus. Infection with

norovirus causes gastroenteritis (inflammation of the stomach and intestines), which most commonly results in diarrhea, vomiting, nausea, and stomach cramping, hence the term "stomach flu" is often used to describe it. However, it is important to note that it is not related to the flu, which is a respiratory illness caused by the influenza virus. Since there are several strains of the norovirus, infection with one strain will not lead to lifetime immunity. Generally protection acquired through infection will be valid for a year. Although viruses by their nature are obligatory parasites and cannot multiply outside the host cell, large particles of norovirus are shed by infected patients. The vomit and fecal matter of the infected patients is considered to be most contagious during the first three days of illness. The virus can still be found for two weeks or more after the patient has recovered. These shed particles cause infection. Food handlers that were sick may have contaminated the food by not exercising strict hygiene, such as thoroughly washing hands with soap and using shared utensils [7, 10].

Toxoplasma gondii is a protozoan parasite that causes the disease toxoplasmosis, which can affect the eyes, liver, lung, and the brain. Serologic testing, biopsy of tissue sections, cerebrospinal fluid, and polymerase chain reaction (PCR) test are routinely used in the diagnosis of toxoplasmosis. It is generally found in warm, tropical and mostly impoverished areas, hence it is also known as the neglected infection of poverty (NIP) disease. Toxoplasmosis is one of the leading causes of death attributed to foodborne illness in the United States. Over 60 million people in the United States carry this parasite, but symptoms are mostly noted only in people with a weakened immune system. These symptoms are characterized by headache, fever, ocular lesions/inflammation, confusion and seizure. In healthy populations, these symptoms are generally not observed because the immune system is able to prevent the parasite from causing illness. However, in some patients symptoms might be characterized as swollen lymph nodes, headaches, fever (similar to mononucleosis) and sore throat. This parasite also exists in the form of microscopic cysts, which can easily be transmitted through ingestion by eating undercooked meat such as vension, lamb and pork, or eating food that was prepared or served using contaminated utensils. Apart from foodborne transmission, it can also be transmitted from mother-to-child (congenital) and animal-to-human (zoonotic). Cats especially are considered to be vectors. Hence, pregnant women are advised to avoid changing cat litter [7, 10, 24, 25, 26].

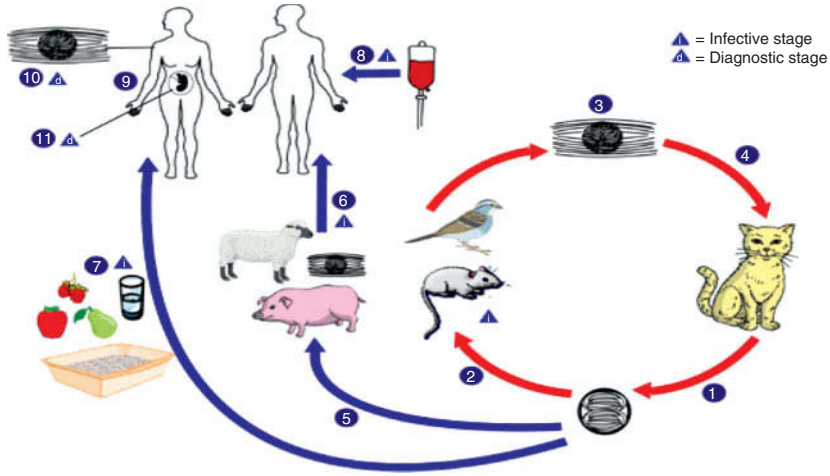

Figure 8.1 Life Cycle of *Toxoplasma gondii* [7]. Source: CDC

Unlike most microbes that cannot proliferate in the cold temperature of a refrigerator, *listeria monocytogenes* thrives at temperature as low as 0°C and in low-pH and high salt conditions. The virulence genes that are encoded by *l.monocytogenes* are thermoregulated and their expression factor is optimal at 37°C. As the bacteria infect the host, the temperature of the host melts the structure and allows translation initiation for the virulent genes. *Listeria monocytogenes* is a gram positive pscyhrophile and facultative anaerobe found in soil and water. Food that is contaminated with *l. monocytogenes* will cause listeriosis. It can be found in raw meats, processing plants, milk, and foods made from raw milk. It can be killed by pasteurization and cooking. The immune system targets the majority of the *listeria* before they are able to cause infection. However in weakened immune systems, these can escape the initial response and are spread through intracellular mechanisms. The common symptoms are fever, stiff neck, confusion, weakness, vomiting, and diarrhea. In pregnant women, during the first trimester listeriosis can induce miscarriages, hence they are advised to refrain from eating certain cheeses that are made from unpasturized milk. Once *l.monocytogenes* gets inside the processing plants, it can survive for years. Currently, serological tests are not reliable in diagnosing the presence of *Listeria*. Diagnosis is confirmed only after isolation of *Listeria monocytogenes* from a normally sterile site, such as blood, or from amniotic fluid or the placenta in a pregnancy setting. In

addition, it can also be isolated using selective media such as blood agar [7, 10, 13, 21]. The plate culture methods are being replaced with rapid biosensors that enable testing of multiple samples for multiple pathogens [14].

Case Scenario – Multistate Outbreak of Listeriosis

In 2011, a multistate outbreak of listeriosis in 139 people was linked to whole cantaloupes. In total, 29 deaths and one miscarriage were reported. In this case, investigators used DNA analysis and pulsed-field gel electrophoresis (PFGE) to determine DNA fingerprint patterns. Cantaloupe samples from the refrigerator of a *Listeria* patient's home, as well as samples obtained from various retail outlets in the preceding week, had the same DNA fingerprints as the *Listeria* that infected various patients. These cantaloupes were linked to Jensen Farms.

Campylobacter are gram negative spirilla that can cause disease in humans and animals. They are generally found in the excrement of infected cows or wild birds, and grow well at the body temperature of a bird. Surface water supplies that are contaminated with infected bird droppings can become breeding pools of *Campylobacter.* Udder hygine and keeping udders free from manure, prevents contamination of milk with *campylobacter.*

Usually chicken flocks are infected with *Campylobacter,* but since birds are well adapted they can harbor it without becoming ill or exhibiting symptoms. In slaughtering plants, when an infected bird is slaughtered *campylobacter* can get transferred from the intestines to the meat. Although *campylobacter* are fragile, every year approximately 124 people die from *Campylobacter* infections. Most people infected with campylobacteriosis recover completely within ten days. Rarely does a *Campylobacter* infection result in long-term consequences. Similar to Salmonellasis, some people may develop arthritis. Others may develop a rare immune disorder known as Guillain-Barré that affects the nerves of the body and causes the immune system to attack the body's own nerves, leading to paralysis. Most of the human illness is caused by one species, called *Campylobacter jejuni*. In 2005, under the U.S. Food and Drug Administration (FDA) National Antimicrobial Resistance Monitoring System (NARMS) retail meat surveillance program, a reported 47% of raw chicken breast tested positive for *Campylobacter* [7, 10].

Listed below are some practices that food handlers and consumers alike should follow when cooking/handling food.

- Thoroughly wash hands with soap and water before starting food preparation, before working with a new food or a new tool, and before serving food.
- Wash fruits and vegetables thoroughly.
- Remove the skins of fruits and vegetables and rinse the knives with water before cutting them.
- Never let raw meat, poultry, or their juices come in contact with other foods.
- Avoid contact with animals or animal feces when cooking or handling food.
- Clean cutting boards, knives, counters and other utensils thoroughly with soap and water and/or bleach solution before and after use.
- Keep separate cutting boards for vegetables and meat and/or dairy products.
- Replace cutting boards, knives, and other utensils frequently.
- Serve prepared foods on clean plates.
- Cook meat thoroughly (heat to 185°F internal temperature).
- Keep hot food hot and cold dishes cold.
- Promptly refrigerate any food that is not consumed immediately after preparation.

Food irradiation, pasteurization, flash pasteurization, freezing, fumigation, and pascalization are common techniques that are used in processing plants to sterilize various food products. While thermization is restricted to milk and various milk products, it inactivates pyschortrophic bacteria.

8.3 Probiotics in Food

In the previous section, the discussion was focused around foodborne pathogens that cause illnesses. This section will focus on microorganisms that are beneficial for the body.

Probiotics are a group of live microorganisms that can help the digestion process and the absorption nutrients found in food. Some

Table 8.3 Number of reported foodborne disease outbreaks and outbreak-associated illnesses, by etiology* and food commodity — Foodborne Disease Outbreak Surveillance System, United States, 2008.

Etiology	Outbreaks (illnesses)							
	Attributed to a single commodity		Attributed to food vehicle containing >1 commodity		Attributed to unknown commodity		Total	
Bacterial								
Salmonella†	40	(`3,690)	24	(734)	53	(536)	**117**	**(4,960)**
Clostridium perfringens	20	(897)	12	(226)	8	(286)	**40**	**(1,409)**
Escherichia coli, Shiga toxin–producing (STEC)§	21	(427)	5	(98)	10	(395)	**36**	**(920)**
Campylobacter¶	17	(538)	2	(6)	6	(71)	25	(615)
Bacillus cereus	7	(70)	7	(50)	1	(2)	**15**	**(122)**
*Staphylococcus enterotoxin***	3	(27)	8	(124)	3	(160)	**14**	**(311)**
Shigella††	0	(0)	0	(0)	6	(170)	**6**	**(170)**
Clostridium botulinum	1	(2)	2	(6)	1	(2)	**4**	**(10)**
Other bacterial	1	(64)	2	(24)	0	(0)	3	(88)

(Continued)

Table 8.3 *(Cont.)*

Etiology	Outbreaks (illnesses)							
	Attributed to a single commodity		Attributed to food vehicle containing >1 commodity		Attributed to unknown commodity		Total	
Listeria§§	2	(28)	1	(5)	0	(0)	**3**	**(33)**
Vibrio parahaemolyticus	1	(2)	0	(0)	0	(0)	**1**	**(2)**
Vibrio other	0	(0)	0	(0)	1	(3)	**1**	**(3)**
Total	**113**	**(5,745)**	**63**	**(1,273)**	**89**	**(1,625)**	**265**	**(8,643)**
Chemical and toxin								
Scombroid toxin/ histamine	11	(53)	1	(2)	0	(0)	**12**	**(55)**
Ciguatoxin	14	(81)	0	(0)	0	(0)	**14**	**(81)**
Cleaning agents	0	(0)	1	(3)	2	(11)	**3**	**(14)**
Heavy metals	0	(0)	1	(2)	1	(52)	**2**	**(54)**
Other chemical	0	(0)	0	(0)	2	(42)	**2**	**(42)**
Mycotoxins	1	(3)	0	(0)	0	(0)	**1**	**(3)**

Etiology	Outbreaks (illnesses)							
	Attributed to a single commodity		Attributed to food vehicle containing >1 commodity		Attributed to unknown commodity		Total	
Paralytic shellfish poison	1	(3)	0	(0)	0	(0)	**1**	**(3)**
Plant/herbal toxins	1	(6)	0	(0)	0	(0)	**1**	**(6)**
Total	**28**	**(146)**	**3**	**(7)**	**5**	**(105)**	**36**	**(258)**
Parasitic								
Cyclospora	3	(66)	0	(0)	0	(0)	**3**	**(66)**
Cryptosporidium	0	(0)	0	(0)	2	(32)	**2**	**(32)**
Giardia	0	(0)	0	(0)	1	(8)	**1**	**(8)**
Total	**3**	**(66)**	**0**	**(0)**	**3**	**(40)**	**6**	**(106)**
Viral								
Norovirus	35	(618)	94	(2,484)	227	(6,073)	**356**	**(9,175)**
Hepatitis A	1	(22)	0	(0)	0	(0)	**1**	**(22)**
Rotavirus	0	(0)	1	(27)	0	(0)	**1**	**(27)**
Other viral	0	(0)	0	(0)	1	(9)	**1**	**(9)**
Total	**36**	**(640)**	**95**	**(2,511)**	**228**	**(6,082)**	**359**	**(9,233)**

(Continued)

Table 8.3 *(Cont.)*

Etiology	Outbreaks (illnesses)							
	Attributed to a single commodity		Attributed to food vehicle containing >1 commodity		Attributed to unknown commodity		Total	
Known etiology¶¶	180	(6,575)	161	(3,791)	325	(7,852)	**666**	**(18,240)**
Unknown etiology***	33	(409)	67	(577)	250	(3,276)	**350**	**(4,262)**
Multiple etiologies	5	(193)	9	(202)	4	(255)	**18**	**(650)**
Total	**218**	**(7,177)**	**237**	**(4,570)**	**579**	**(11,383)**	**1,034**	**(23,152)**

* If at least one etiology was laboratory-confirmed, the outbreak was considered to have a confirmed etiology. If no etiology was laboratory-confirmed, but an etiology was reported based on clinical or epidemiologic features, the outbreak was considered to have a suspected etiology.

† *Salmonella* serotypes accounting for more than five reported outbreaks included: *Enteriditis* (30 outbreaks), *Typhimurium* (18), Heidelberg (eight), and Braenderup (six).

§ STEC O111 (one confirmed outbreak), STEC O157:H7 (32 confirmed outbreaks), and STEC O157:NM(H-) (three confirmed outbreaks).

¶ *Campylobacter coli* (one confirmed outbreak, no suspected outbreaks), *Campylobacter jejuni* (15 confirmed outbreaks, four suspected outbreaks).

** *Staphylococcus aureus* (six confirmed outbreaks, five suspected outbreaks) and *Staphylococcus* unknown (three suspected outbreaks).

†† *Shigella sonnei* (six confirmed outbreaks, no suspected outbreaks).

§§ *Listeria monocytogenes* (three confirmed outbreaks, no suspected outbreaks).

¶¶ The denominator for the etiology percentages is the known etiology total. The denominator for the known etiology, unknown etiology, and multiple etiologies percentages is the total.

*** An etiologic agent was not confirmed or suspected based on clinical, laboratory or epidemiologic information.

Source: CDC [7].

of these are part of the normal flora. Probiotics are different from *prebiotics* which are non-digestible food ingredients that stimulate bacterial activity in the digestive system; an example of this is insoluble fiber [20].

Lactobacilli and *bifidobacteria* are two very common probiotic microbes. *Lactobacilli* is a type of lactic acid bacteria. These types of bacteria are commonly found in yogurt, milk, cheese and dairy products. *Bifidobacteria* are dominant in the gut flora of babies that are fed with breast milk and are known to cause displacement of proteolytic bacteria causing the disease. Probiotic microbes can act directly with the gastrointestinal tract by interacting with the existing intestinal flora or they can interact with the intestinal mucus layer and epithelium. Some can also have an impact outside the gastrointestinal system on other organs such as the liver and brain. Live probiotic cultures are found in fermented dairy products such as soft cheese and probiotic-fortified foods. However, tablets, capsules, powders and sachets containing the bacteria in freeze dried form are also available [15, 17, 18, 20, 37, 38, 39].

Initially culture-based methods were used to evaluate the microbial diversity, however, the drawback of this method is that a significant amount of microbes cannot be cultured. The collective genome of human intestinal microbiota contains ~3.3 million microbial genes compared to ~24,000 genes in the human genome. It is clear that the presence of this intestinal microbiota plays a significant role in host cell interactions [15, 17, 18, 20].

Genetic sequencing that targets the conserved 16S ribosomal RNA (rRNA) genes found in prokaryotes sequences of these bacteria allows the analysis of their genetic diversity. Metagenomic studies or population genetics can provide information on the diversity of the genes encoded by the intestinal microbiota. There are various molecular biology tools such as quantitative polymerase chain reaction (qPCR), temperature or denaturing gradient gel electrophoresis (TGGE or DGGE), terminal-restriction fragment length polymorphism (T-RFLP) and fluorescent *in situ* hybridization (FISH) that can be used to study the microbial diversity of intestinal microbes [34].

The drawbacks of probiotics are increased susceptibility to system infections, gene transfer, deleterious metabolic activities, and excessive immune stimulation. The World Health Organization (WHO) and Food Agriculture Organization of United Nations (FAO) provide guidelines on evaluating the safety of probiotics in

food. These include viability of the microorganisms in relation with the shelf life of the product, identification of the strain by genotypic and phenotypic methods and depositing the strain in international culture collection, and *in-vitro* tests and animal studies. The *in-vitro* and animal tests evaluate the probiotics for the following factors:

- Resistance to gastric acidity
- Bile resistance
- Adherence to mucus/human epithelial cells
- Antimicrobial activity against pathogenic bacteria
- Ability to reduce pathogen adhesion
- Bile salts hydrolase activity

Research suggests mixed results and lack of efficacy of probiotics in human studies. The European Food Safety Authority has rejected over 200 claims on probiotics in Europe due to insufficient research and lack of conclusive evidence. The new trends in research are focusing on the potential health benefits of multiple probiotic strains as a health supplement as opposed to a single strain [15, 17, 20].

8.4 Genetically Modified (GM) Foods – Friends or Foe?

The central dogma of molecular biology states that DNA will undergo transcription to produce pre-mRNA, which will undergo further processing and lead to mRNA which undergoes translation and produces protein. In a nutshell, genes code for protein. The structure of the protein will determine its function. When this protein is expressed it results in the production of a phenotype [16].

$$\text{DNA} \longrightarrow \text{pre-mRNA} \longrightarrow \text{mRNA} \longrightarrow \text{Protein}$$

Foods that have been modified genetically using recombinant DNA technology to introduce desirable traits are known as genetically modified (GM) foods. The objective of GM foods is to boost their nutrition value, increase production or increase their resistance. Soybean, cotton, maize and canola are the dominant worldwide GM crops. It allows selected individual genes to be transferred from one organism into another, also between non-related species

In GM plants, a novel gene from a different species is inserted into the specific location in the plant genome; expression of this gene results in the formation of a novel protein, which causes a change in the phenotype. This technique can be used to increase nutritional content of foods or transmit nutrients in crops that are otherwise nutrient-poor [9, 16, 22, 30].

Case Scenario – Golden Rice

Malnutrition is the leading cause of various diseases in developing countries. People living in remote areas cannot afford a nutritious diet. People in remote rural areas of South-East Asia generally survive on rice. Rice is rich in carbohydrates but has a very low content of micronutrients. VAD (vitamin A deficiency) alone results in approximately 1.15 million deaths due to diseases that are caused directly due to this deficiency. Golden Rice is genetically engineered rice with beta carotene (precursor to Vitamin A). Golden Rice has been genetically engineered with two foreign genes—one from a soil bacterium (*Erwinia uredovora*) and one from maize or daffodil (*Narcissus pseudonarcissus*). These two genes code for enzymatic products that result in formation of beta-carotene, which is then converted to Vitamin A in mammals. Evidence indicates that Golden Rice should be able to provide the full recommended daily intake of provitamin A in rice-eating societies. These genes encode products that have enzymatic activities that are usually present in green plant tissues and many flowers, where beta-carotene and carotenoids are produced [9, 16, 30].

Often times proteins coded from these novel genes may have a similar structure to proteins that are known to produce allergic reactions in humans. It is crucial to examine the toxicity, allergenicity and loss of nutritional value. Novel genes that have been introduced into a plant can also interact with existing genes, causing shifts in a plant's metabolism. There is also a likelihood that this new gene may interfere with different plants. When a new gene is transferred to a plant, one cannot automatically rule out the possibility of unforeseen "side effects." This has to do with the fact that a new gene can interact with existing genes. For instance, a new gene could deactivate an existing gene, thereby causing shifts in a plant's metabolism. In certain cases, such mutations can potentially impact human health [1, 16, 17, 30, 35].

The risk assessment of GM plants evaluates the following factors [9, 16, 31]:

- characteristics of the donor and recipient organisms;
- genes that have been inserted and expressed;
- stability of the inserted gene;
- development of antibiotic resistance;
- any unintended consequences of the genetic modification;
- potential toxicity and allergenicity of gene products and metabolites;
- compositional, nutritional, safety and agronomic characteristics;
- the influence of food processing on the properties of the food or feed;
- changes in nutritional value and possible changes in dietary intake;
- long-term impact on the ecosystem; and
- the possibility of these gene being introduced in other crops.

Chemical analysis tests are conducted to evaluate the composition of the GM plant and feeding tests are conducted. In feeding tests, animals are fed products of GM plants and evaluated for toxic and allergic effects. Organ effects are also observed. In chemical analysis, by using chromatography techniques, changes in plant ecosystems can be detected by measuring the light reflected from plant foliage. Airborne satellite surveillance can be used to monitor changes in this reflected light for evaluating plant growth and identifying disease conditions. The image data from this surveillance is transferred to a computer program that runs algorithms and analyses of pest infestation in all varieties of crops, and distinguishes GM crop varieties from neighboring non-GM crops. This process does not replace field inspection, but is rather used to complement field inspection by proactively identifying potential problems [16, 30].

Currently, the two dominant genetically modified agronomic traits that are extensively used are herbicide tolerance (HT) and insect resistance (mostly in the form of *Bt* crops). Herbicide tolerance is achieved through the introduction of a gene from a bacterium conveying resistance to some herbicides. In situations where weed pressure is high, the use of such crops has resulted in a reduction in the quantity of the herbicides used [30].

Case Scenario – StarLink Corn

StarLink corn is a GM *Bt* corn that was approved by the Environmental Protection Agency (EPA) to be sold only as animal feed or for industrial purposes. The Washington-based group, Genetically Engineered Food Alert, discovered traces of StarLink corn genetically altered by Aventis SA in taco shells made by Taco Bell and in other corn products. StarLink was a corn variety modified to produce a *Bacillus thuringiensis* (Bt) endotoxin, Cry9C. The CDC investigated 51 reports of possible adverse reactions to corn that occurred after the announcement that StarLink, allowed for animal feed, was found in the human food supply. Allergic reactions were not confirmed, but tools for postmarket assessment were limited. The physical features of StarLink corn were very similar to yellow corn. StarLink made its way into the human food supply through the distribution system in the processing plants. Apart from the financial loss, this incident caused a loss of trust in GM foods [30].

8.5 Bioavailability of Nutrients

The old saying, "you are what you eat," does hold some truth to it. Apart from the savory taste and aromatic flavors, the most important component of consuming foods is to provide our body the nutrients it needs. The food that we eat provides our body with energy in the form of calories. Apart from energy, food also provides macronutrients and micronutrients.

Macronutrients are the nutrients that our body needs in large amounts to conduct various cellular processes such as energy production, cellular growth and repair, and immune system response. Macronutrients include carbohydrates, proteins, fats and fatty acids. They also include vitamins, essential elements and phytochemicals. When we consume food, it gets broken down and processed by several enzymatic reactions in our body; the nutrients are released and absorbed by our body. The small intestine contains several folds that are covered with microscopic projections called microvilli. Specialized cells enable absorbed materials to cross the mucosa into the blood and are carried off in the bloodstream to various parts in our body for storage or chemical processing. However, not all nutrients in a particular food will be absorbed into our body. Bioavailability of nutrients refers to the absorption and

utilization of nutrients from food by our body [19]. Just because a cereal is labeled as containing a 100% daily intake value of Vitamin A, it does not mean that our body will be able to absorb this in its entirety.

Bioavailability or absorption of nutrients from food is a complex process as it is influenced by age, gender, health condition and other factors. For example, an individual that suffers from lactose intolerance will not absorb calcium as readily because lactose enhances absorption and lactose-free diets result in lower calcium absorption and may predispose the individual to inadequate bone mineralization. The various factors that influence absorption of nutrients in our body are: the amount of nutrient, chemical form of the nutrient, the health of the individual, genetic variability, nutrient-nutrient interactions, nutrient-drug interactions, and nutrient-environmental interactions [19, 27, 28, 29, 32, 38].

There are two main types of vitamins—fat soluble and water soluble. Vitamins with lipophilic structures are readily absorbed when delivered in the presence of fat from the small intestines. These vitamins are stored in the liver and adipose tissues and an accumulation of these vitamins can lead to toxicity. These fat soluble vitamins are absorbed readily when consumed with foods that have a good fat content as opposed to water. Whereas, water soluble vitamins such as vitamin C, thiamin, riboflavin, niacin, vitamin B_6 (pyridoxine, pyridoxal, and pyridoxamine), folacin, vitamin B_{12}, biotin, and pantothenic acid absorb readily when consumed with water and do not accumulate in the liver or the adipose tissues; these are readily excreted. Likewise, prolonged chewing reduces food intake. The phytates, phosphates, and tannins in excess may slow down the absorption of various nutrients. Vitamin C and citric acid enhance the absorption of trace elements such as iron and also boost absorption of minerals such as calcium and trace elements such as iron. Raw carrots and spinach are good sources of dietary fiber and carotenoids and cooking them opens up their cell walls and enables the body to extract a higher amount of carotenoids [3, 4, 27, 28, 29, 32, 37, 39].

Fortification is a process in which vitamins and minerals are added to foods to increase their nutritional value while biofortification is used to improve the micronutrient quality of staple crops by combining biotechnology with traditional breeding [17].

The sequencing of human genome and novel sequencing methods have given birth to the field of nutritional genomics. In 2005,

the University of Tokyo launched a nutrigenomics database that serves as a repository for microarray gene expression data for nutritional scientists. Nutritional genomics or nutrigenomics evaluates how foods impact our genes and the genetic differences that can impact the way individuals respond to nutrients in the food. Recent evidence suggests that 9p21 genetic variant that is a strong marker for heart disease can be mitigated by consuming a diet high in fruits and vegetables. This research suggests there may be a significant interplay between genes and diet in cardiovascular disease. Furthermore, this research has paved the road for more research associating the role of diet in influencing expression of disease-specific genetic variants [2–4, 12, 17–19, 23, 26, 27–29, 31, 36–39].

8.6 Food Safety Regulations

Everyone from the producer to the consumer play an important role in food safety. In the United States, the oldest laws dealing with meat inspection were enacted in 1906. These older laws simply focused on visual inspection of live animals to ensure that they were healthy and the removal of spoiled meat. In July 1996, the Food Safety and Inspection Service created Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems, a rule which further strengthened food safety by requiring all federally inspected meat and poultry farms to develop a "strategy to identify critical points in the processing of animals and food products whereby products are likely to become contaminated by pathogenic organisms, establish critical limits for contaminants, establish corrective actions to prevent contamination and monitor the plant procedures." For example, it is a mandatory practice for meat processing plants to check the control of fecal contamination by testing for *E.coli*. In 2011, the FDA Food Safety Modernization Act (FSMA) was enacted. This law provides the FDA with additional authority that enables it to focus on prevention of contamination as opposed to simply responding to contamination. FoodNet is an active surveillance system established by the CDC. It monitors the incidence of various foodborne illnesses. On an international forefront, the WHO Food Safety Programme assists national authorities in the identification of foods that should be subject to risk assessment. The Codex Alimentarius Commission (Codex) is the joint FAO/WHO body responsible for compiling the standards, guidelines

and recommendations that constitute the Codex Alimentarius: the international food code. Codex is developing principles for the human health risk analysis of GM foods [5–7, 10–12, 23, 24, 31, 32].

8.7 Conclusion

Food science is constantly evolving with new advances in food safety, and the production of GM plants. Ultimately, this is a global responsibility and everyone from the producers to the consumers plays an active role. The challenges of readily available food products, climate change, foodborne pathogens and bioavailability of nutrients, coupled with the development of sophisticated genetic recombination techniques and novel testing methods to detect foodborne pathogens, provide us with the tools we need to ensure the supply of safe and nutritious food.

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9

Transformations of Food Flavor Due to Industrially Processing of Elaboration

Romina P. Monasterio

Instituto de Biología Agrícola de Mendoza (IBAM) CCT-CONICET Mendoza, Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza, Argentina

Abstract

Flavor is a multifaceted appreciation of the total sensation perceived when any food or drink is consumed. The flavor of food is the most important sensory attribute affecting the acceptance and preference of consumers. A flavor is the substance which may be only a chemical entity or a blend of chemicals (natural or synthetic), whose primary purpose is to provide all or part of the particle flavor or effect to any food or other product taken into the mouth. At one time, researchers in both academic and industrial settings viewed flavor as principally aroma with only minor importance given to the contribution of taste and somatosenses.

The new technological processes used in food elaboration are other important topics in food flavor, making it a dynamic subject matter. The multitude of interactions between all components and environmental factors (such as temperature, water content, etc.) give the final sensorial quality to food and beverage.

The chemical reactions that contribute in food flavor are principally Maillard reaction, reactions from lipids and all compounds formed through fermentation.

Keywords: Flavor, Maillard reaction, fermentation, industrial process, threshold, chemical reaction

**Corresponding author*: rmonasterio@mendoza-conicet.gob.ar

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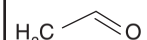
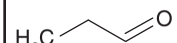
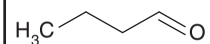
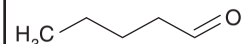
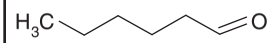
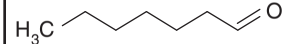
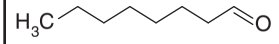
9.1 Introduction

Flavor is a multifaceted appreciation of the total sensation perceived when any food or drink is consumed. The flavor of food is the most important sensory attribute affecting the acceptance and preference of consumers [1]. Standards organizations in many countries define flavor as a total impression of odor, taste, tactile, kinesthetic, temperature and pain sensations perceived through tasting. It is widely accepted that flavor includes the aromatics, such as olfactory perceptions caused by volatile substances; the tastes, such as gustatory perceptions (salty, sweet, sour and bitter) caused by soluble substances; and the chemical feeling factors that are perceived as astringency, spicy hot, cooling, and metallic flavor, stimulating the nerve ends in the membranes [1, 2]. Currently, a complete flavor experience depends on the combined responses of our senses and the cognitive processing of these inputs. As above, numerous sensory inputs are processed by the brain to result in flavor perception [3]. A flavor is the substance which may be an only chemical entity or a blend of chemicals (natural or synthetic) whose primary purpose is to provide all or part of the particle flavor or effect to any food or other product taken into the mouth. Research about the multimodal aspect of flavor perception is carried out for multidisciplinary groups by understanding this complex concept. At one time, researchers in both academic and industrial settings viewed flavor as principally aroma with only minor importance given to the contribution of taste and somatosenses [4].

Flavors represent important challenges in terms of process engineering because they cover a very broad range of sensory and thermophysical characteristics. Besides, they are sometimes unstable and are perceived by human beings on the basis of very complex, extremely nonlinear mechanisms [5, 6]. Commercial flavorings are complex mixtures of solvents, pure flavoring agents and natural isolates, which in turn consist of flavoring agents. Similar examples are listed in Table 9.1, where the physicochemical properties and aroma threshold values are shown. The aroma threshold value is the lowest concentration of a certain odor compound that is perceivable by the human sense of smell. The threshold of a chemical compound is determined in part by its shape, polarity, partial charges and molecular mass.

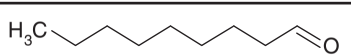
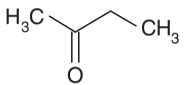
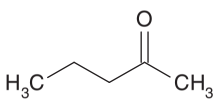
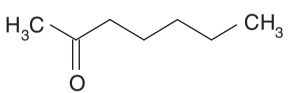
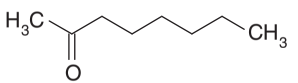
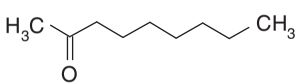
The chemical reactions that contribute to food flavor are principally Maillard reaction, reactions from lipids and all compounds

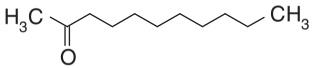
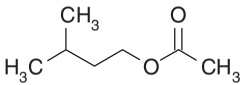
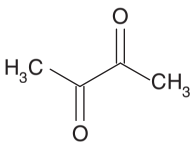
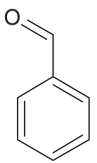
Table 9.1 Physicochemical properties and others characteristics of flavor compounds.

Name (CAS Nº)	Structure (MW)	T_b (ºC)	Log P_{ow}	Sensory description	Threshold	Ref.
Acetalde-hyde (75-07-0)	 (44.05)	20.2	0.43	Green, fresh	400 ppb	[7]
Propanal (123-38-6)	 (58.08)	48.8	0.83	Ethereal, caramel, cooked, brothy	170 ppb	[7]
Butanal (123-72-8)	 (72.11)	74.8	0.88	Fatty, cocoa, green	70 ppb	[8]
Pentanal (110-62-3)	 (86.13)	103	1.44	Bitter almond	2.67 ppm	[9]
Hexanal (66-25-1)	 (110.16)	130	1.78	Tallowy, green leaf	4.5 ppt	[10]
Heptanal (111-71-7)	 (114.19)	153	2.50	Fatty rancid; fermented, fruit-like odor	0.9 ppt -0.23 ppm	[11]
Octanal (124-13-0)	 (128.22)	171	3.03	Oily, fatty, soapy	0.4 ppt -0.02 ppm	[8]

(Continued)

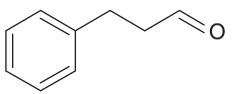
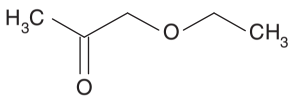
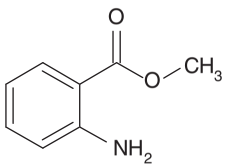
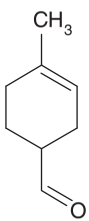
Table 9.1 *(Cont.)*

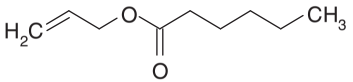
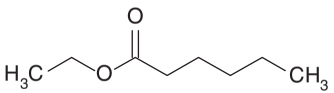
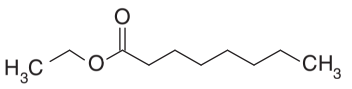
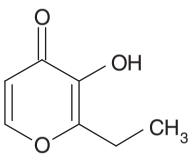
Name (CAS Nº)	Structure (MW)	T_b (ºC)	Log P_{ow}	Sensory description	Threshold	Ref.
Nonanal (124-19-6)	 (142.20)	195	3.56	Tallow, fatty, fruity	2.6 ppt	[12]
Butan-2-one (78-93-3)	 (72.11)	79.6	0.26	Chocolate	50 ppm	[10]
Pentan-2-one (107-87-9)	 (86.14)	101	0.84	Fruity, banana	2.3 ppm	[9]
Heptan-2-one (110-43-0)	 (114.19)	149	1.98	Spicy, blue cheese	650 ppb	[8]
Octan-2-one (111-13-7)	 (128.22)	173	2.53	Floral, green	190 ppb	[10]
Nonan-2-one (821-55-6)	 (142.23)	194	3.03	Floral, green	190 ppb	[10]

Name (CAS Nº)	Structure (MW)	T_b (ºC)	Log P_{ow}	Sensory description	Threshold	Ref.
Undecan-2-one (112-12-9)	 (170.30)	231	4.09	Waxy	–	[11]
Isoamyl acetate (193-92-2)	 (130.19)	142	2.26	Fruit, banana sweet or pear	1–1.6 ppm	[13]
Diacetyl (431-03-8)	 (86.08)	88	-1.34	Buttery, butterscotch	1–2.5 ppm	[9]
Benzalde-hyde (100-52-7)	 (106.12)	178	1.48	Bitter almond	1.5 ppm	[9]

(Continued)

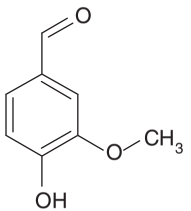
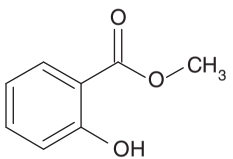
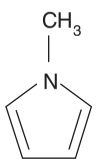
Table 9.1 *(Cont.)*

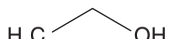
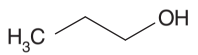
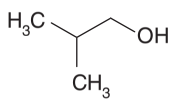
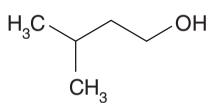
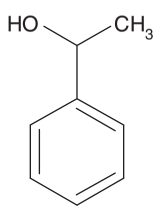
Name (CAS Nº)	Structure (MW)	T_b (ºC)	Log P_{ow}	Sensory description	Threshold	Ref.
Cinnamic aldehyde (104-55-2)	 (136.16)	248	1.90	Cinnamon	–	[7]
Ethyl propanoate (105-37-3)	 (102.13)	98	1.21	Fruity, sweet fruity rum juicy fruit grape pineapple	–	[8]
Methyl anthranilate (134-20-3)	 (151.16)	256	2.04	Grape	–	[8]
Limonene (5989-27-5)	 (136.24)	176	4.2	Orange	–	[11]

Name (CAS Nº)	Structure (MW)	T_b (ºC)	Log P_{ow}	Sensory description	Threshold	Ref.
Allyl hexanoate (123-68-2)	 (156.22)	190	3.12	Pineapple	–	[12]
Ethyl hexanoate (123-66-0)	 (144.21)	166	2.83	Sour apple	0.14 ppm	[13]
Ethyl octanoate (106-32-1)	 (172.26)	206	3.90	Sour apple	0.17 ppm	[13]
Ethyl maltol (4940-11-8)	 (140.14)	289	0.61	Sugar, cotton candy	7.1–13 ppm	[9]

(Continued)

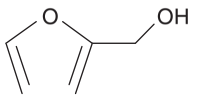
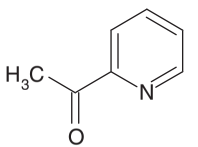
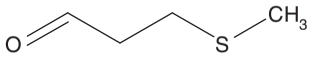
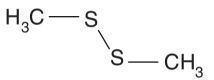
Table 9.1 *(Cont.)*

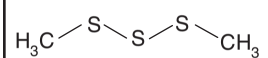
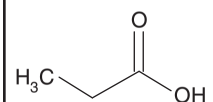
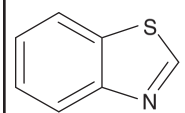
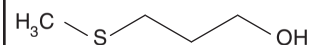
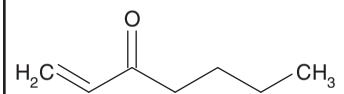
Name (CAS Nº)	Structure (MW)	T_b (ºC)	Log P_{ow}	Sensory description	Threshold	Ref.
Vanillin (121-33-5)	 (152.14)	285	1.21	Vanilla	25 ppb	[12]
Methyl salicylate (119-36-8)	 (152.14)	222	2.55	Wintergreen	–	[9]
1-methyl pyrrole (96-54-8)	 (81.11)	112	1.43	Woody	–	[7]

Name (CAS Nº)	Structure (MW)	T_b (ºC)	Log P_{ow}	Sensory description	Threshold	Ref.
Ethanol (64-17-5)	 (46.07)	78	-0.14	Alcoholic	–	[8]
Propanol (71-23-8)	 (60.09)	97	0.33	Solvent-like	21 ppm	[13]
Isobutanol (78-83-1)	 (74.12)	108	0.76	Alcoholic	10-100 ppm	[13]
Isoamyl alcohol (123-51-3)	 (88.14)	131	1.22	Fruity, sweet	60-65 ppm	[13]
Phenyletha-nol (98-85-1)	 (122.16)	204	1.41	Rose, floral	100 ppm	[13]

(Continued)

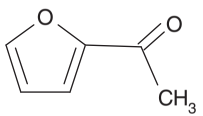
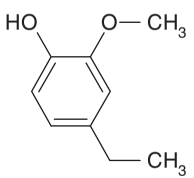
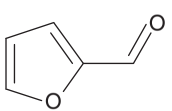
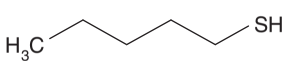
Table 9.1 *(Cont.)*

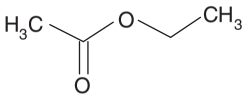
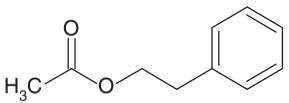
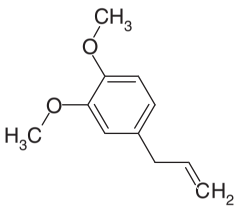
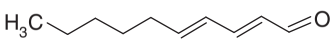
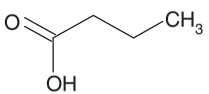
Name (CAS Nº)	Structure (MW)	T_b (ºC)	Log P_{ow}	Sensory description	Threshold	Ref.
Furfuryl alcohol (98-00-0)	 (98.19)	170	0.21	Burnt, oily	5 ppm	[10]
2-acetyl pyridine (1122-62-9)	 (121.13)	189	0.85	Fatty, oily, popcorn, tobacco	–	[9]
Methional (3268-49-3)	 (104.17)	165	0.71	Meaty, onion	–	[14]
Methane-thiol (74-93-1)	H_3C——SH (48.11)	6.10	0.72	Cabbage, garlic	–	[14]
Dimethyl disulphide (624-92-0)	 (94.19)	109	1.77	Alliaceous, onion	–	[14]

Name (CAS Nº)	Structure (MW)	T_b (ºC)	Log P_{ow}	Sensory description	Threshold	Ref.
Dimethyl trisulphide (3658-80-8)	 (126.26)	165	2.93	Alliaceous, etheral, fresh, green, onion	3 ppb	[12]
Propionic acid (propanoic acid) (79-09-4)	 (74.07)	140	0.19	Pungent, rancid, sour milk	2 ppm	[11]
Benzo-thiazole (95-16-9)	 (135.18)	231	2.17	Sulfureous, rubbery, vegetative, cooked, brown, nutty, coffee-like and meaty	–	[7]
3-methylsulfanylpropan-1-ol (Methionol) (505-10-2)	 (106.2)	194	0.40	Sweet soup, meat-like	1–3 ppm	[15]
1-octen-3-one (4312-99-6)	 (126.19)	174	2.17	Mushroom	0.03–0.12 ppt	[8]

(Continued)

Table 9.1 *(Cont.)*

Name (CAS Nº)	Structure (MW)	T_b (ºC)	Log P_{ow}	Sensory description	Threshold	Ref.
2-acetyl furan (1192-62-7)	 (110.10)	173	0.52	Balsamic	80 ppm	[12]
4-ethyl guaicol (2785-89-9)	 (152.19)	235	2.18	Spicy	50 ppb	[7]
Furfural (98-01-1)	 (96.08)	161	0.71	Bready, sweet, woody, almond, bread-like, caramellic	5 ppb	[8]
1-penta-nethiol (110-66-7)	 (104.21)	124	2.74	Sulphurous, fatty/roast meat	0.8 ppb	[9]

Name (CAS Nº)	Structure (MW)	T_b (ºC)	Log P_{ow}	Sensory description	Threshold	Ref.
Ethyl acetate (141-78-6)	 (88.10)	77	0.71	Solvent-like	21-30 ppm	[13]
Phenyl ethyl acetate (103-45-7)	 (164.20)	238	2.30	Rose	3.0 ppm	[13]
Eugenol (93-15-2)	 (178.22)	254	2.97	Clove, spicy	–	[11]
2,4-decadienal (2363-89-4)	 (152.23)	244	3.18	Buttery, fatty, orange	–	[8]
Butyric acid (107-92-6)	 (88.10)	162	0.79	Cheesy	25 ppm 0.6 ppm	[12]

formed through fermentation. These reactions will be explained in following sections.

9.2 Aroma Compounds

Flavor isolation and analysis is one of the most difficult tasks by an analytical chemist. This task is difficult because aroma compounds (flavor components) include a large number of chemical classes. If the aroma compounds were comprised of one or just a few classes of compounds, isolation methods could focus on characteristic molecular properties of a given group of compounds. Other difficulties during the flavor components analysis are the low concentrations at which these analytes may be present in food. In this sense, laboratory instrumentation is not as sensitive to a lot of odors as is the human olfactory system. Furthermore, as can be seen in Table 9.1, the number of flavor components can be very large and their chemical properties different, so the chemist must attempt to effectively extract and concentrate aldehydes, alcohols, acids, amines, ketones, carbonyls, heterocyclics, aromatics, gases, nonvolatiles (or nearly so), etc.

Finally, a problem that makes the study of flavor difficult is its instability during analysis and sample preparation. Foods are a dynamic system that can change even while stored awaiting analysis. Consequently, the all analytical process must be very controlled to obtain representative and reproducible results. If possible, the flavor isolation process must be strong enough to extract the analytes and at the same time be sufficiently careful to not modify them. Unfortunately, once we have considered each of the points above and attain some instrumental result of the flavor compounds in a certain food, we are left with the enormous question of attempting to determine the importance of each compound to the perceived flavor. During the past 50 years this topic has been the subject of immeasurable research articles.

9.3 Chemical Reactions that Contribute to Food Flavor

Man has been interested in cooked flavors starting with the evolution of the human species. Man is the only representative of the

animal kingdom that has established the practice of cooking food before eating it. The use of fire (and later other heat sources), fermentation and other processes to render a raw palatable material is one of man's higher intellectual achievements. Chemistry in its different discipline allows for the further development of our basic understanding of the components that arise during processing or home preparation of foods and the involved mechanisms. Examples of foods where this occurs include chocolate, coffee, sauerkraut, yogurt, meats, baked goods, and deep fat fried foods. The main routes for flavor development in these foods are fermentation, non-enzymatic browning, and thermal oxidations of fats.

Diversity of variables, such as yeast strain used, fermentation conditions and composition of fermentation medium, temperature and water activity during a Maillard reaction and lipids reaction are known to affect flavor compounds production. So, it is clearly shown that transformations of food flavor during industrial processing, elaboration and storage are numerous.

9.3.1 Maillard Reaction

The Maillard reaction is of the greatest importance for quality of foods, in particular for heated foods. It induces browning of foods, has a nutritive value effect, can have toxicological implications (such as the formation of acrylamide), can produce antioxidative components, ultraviolet absorbing intermediates, high-molecular-weight melanoidins, and it also has a large effect on flavor. In the Maillard reaction, reducing sugars (glucose and fructose) interact with amino acids at high temperature to produce dark-colored products (carbonyl group with nucleophilic amino group) [7]. This reaction is accelerated in alkaline medium due to amino group deprotonation and, hence, nucleophilicity increases. The Maillard reaction was first described by the French chemist *Louis Maillard* in 1913 [8].

Frequently, this non-enzymatic reaction is divided into three steps: condensation of amino groups and reducing sugars; sugar fragmentation products and release of the amino group, and finally different reactions in which amino groups participate again. The first step starts with the condensation between an amino group and a reducing sugar, obtaining a N-substituted glycosylamine in the case of an aldose sugar, which rearranges to form the so-called Amadori product (or Heyns product if the reducing sugar is

a ketose). The intermediate stage starts from the Amadori/Heyns product, leading to sugar fragmentation products and release of the amino group. The degradation of the Amadori product is dependent on the pH of the system. At pH 7 or below, it undergoes mainly 1,2-enolization with the formation of furfural (when pentoses are involved) or hydroxymethylfurfural (when hexoses are involved). At pH greater than 7 the degradation of the Amadori compound is thought to involve mainly 2,3-enolization, with reductones, such as 4-hydroxy-5-methyl-2,3-dihydrofuran-3-one, and different fission products, including pyruvaldehyde, acetol and diacetyl. All these compounds are highly reactive and take part in further reactions. Carbonyl groups can condense with free amino groups, which results in the incorporation of nitrogen into the reaction products. The reaction known as Strecker degradation also occurs, in this reaction dicarbonyl compounds will react with amino acids with the formation of aldehydes and a-aminoketones. The last step, in which amino groups take part, leads to all sorts of dehydration, fragmentation, cyclization, retroaldolizations, rearrangements, isomerizations and polymerization reactions. In the final stage the compounds known as melanoidins appear. These lead to the formation of brown nitrogenous polymers and co-polymers. It should also be cited that sugar degradation reactions in the absence of amino groups (caramelization) resulted in similar products, but the amino group acts as a catalyst in the Maillard reaction, so that this reaction results in a faster reaction and higher amounts of very reactive intermediate products. The many possible reaction paths depend strongly on temperature, pH and nature of the reactants (i.e., type of proteins, sugar or amino acid). In Figure 9.1 different pathways of Maillard reaction are shown.

9.3.1.1 Formation of Flavor Compounds in the Maillard Reaction

The potential reactants of Maillard reaction are sugar, ascorbic acid, amino acids, thiamine, and peptides. These compounds are present in the majority of foods, so the Maillard reaction commonly occurs when these foods are subjected to a thermal process [9]. Flavor compound formation in the Maillard reaction depends on the type of sugars and amino acids involved (determining the type of flavor compounds formed), and on reaction temperature, time, pH and water content (influencing the kinetics of the reaction) [10, 11, 12]. The products of Maillard reaction are aldehydes,

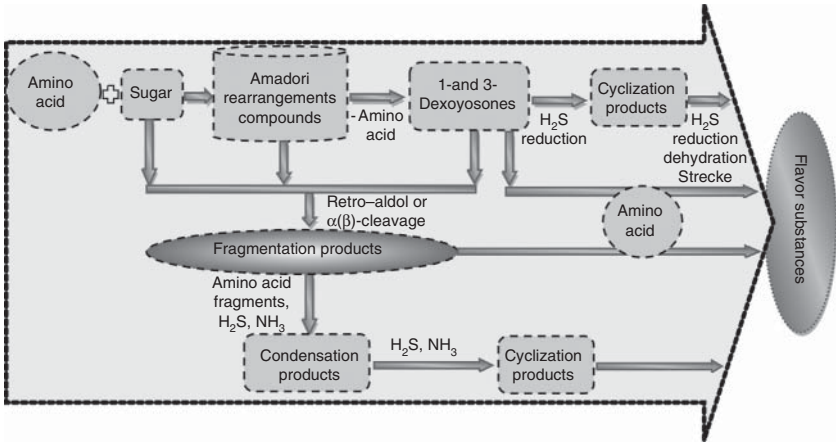


Figure 9.1 Schematic diagram of different pathways of Maillard reaction.

acids, sulfur compounds (e.g., hydrogen sulfide and methanethiol), nitrogen compounds (e.g., ammonia and amines), and heterocyclic compounds such as furans, pyrazines, pyrroles, pyridines, imidazoles, oxazoles, thiazoles, thiophenes, di- and trithiolanes, di- and trithianes, and furanthiols [13, 14]. Higher temperature results in production of more heterocyclic compounds, among which many have a roasty, toasty, or caramel-like aroma [15].

Some examples of how the type of amino acid and sugar can affect the flavor are the meat-related flavor compounds. They are mainly sulphur containing compounds, derived from cysteine and ribose (coming from nucleotides), while the amino acid proline gives rise to typical bread, rice and popcorn flavors. Many Strecker aldehydes themselves are important for food flavor, but also all kinds of reaction products derived from them. Most research about Maillard compounds and its flavor study what mix of sugar-amino acids, and/or sugar-peptides produce each flavor. With peptides and proteins, and in the absence of free amino acids, the Strecker reaction cannot take place, and this has consequences for flavor generation. Another example of the importance of the Maillard reaction are components formed during the malt roasting process, such as Strecker aldehydes [16], nitrogen heterocycles [17], sulfur heterocycles [18], and volatile phenols [19]. These are known to have an important impact on the flavor of beers made with dark specialty malts [17].

9.3.1.2 Kinetics and Factors Influencing the Maillard Reaction

The Maillard reactions have a very complex kinetic due to the numerous reaction paths and effect of processing conditions. The traditional approach of applying simple kinetics (zero-, first-, or second-order behavior) is not very helpful because it pertains to only one single step. Same authors have an interesting approach regarding kinetics of flavor compound formation, such as Jousse *et al.* This research group was able to do a compilation of different literature and reached a generic model [10, 15]. Neverthless, the Maillard reaction has many products (color, flavor and other interesting compounds such as acrylamide); so, it seems that a coupling of the various models that are now published in literature is the next step in developing a tool for product and process design. With such a tool, it should be possible to predict the formation of desired flavor and colored compounds, as well as that of undesired compounds (which could also be flavor compounds).

The reactions that lead to the production of any given flavor are extremely specific. Thus, the reactants environment and heating conditions must be selected carefully to produce the desired flavor. In addition, in an effort to be able to know the role of a given compositional factor, environmental or processing variable, simple model systems have been used. Unfortunately, the addition of an extra component (e.g., another salt component or amino acid) or small change in environment or process can greatly change the reaction rate and direction. Thus, it is particularly difficult to translate model system data to real food data. As a result, its knowledge is incomplete, especially in terms of understanding this reaction in complex food systems.

As mentioned previously, the parameters that influence the flavor formation are numerous. Among them are temperature (and heating time), moisture, system composition, water activity, pH and buffer system, oxidation state, etc.

The temperature and duration of heating were studied as an important variable by Maillard himself [8]. Maillard reported that the rate of the reaction increases with temperature and, besides, it is one of the most important parameters that influence flavor compounds. Subsequently, many workers have confirmed this observation [9, 20, 21]. An increase in temperature leads to enhanced reactivity between the amino group and the sugar. The Arrhenius

equation can describe the temperature dependence with reaction rate constant k ($k = A \exp(-Ea/RT)$ where k is the rate constant; A the frequency factor; Ea the activation energy; R the gas constant and T is the temperature (K) [22]. The influence of temperature on flavor formation may be understood better by recalling that each particular pathway of flavor formation has its own activation energy.

Obviously, another important variable in Maillard reaction is the influence of system composition. Different authors had reported the importance of types and concentration of reactants, about, for example, the rate of the reaction. In general, the rate of the reaction is affected by sugar composition as follows: pentoses (xylose or arabinose) > hexoses (glucose or fructose) > disaccharides (lactose or maltose) > trisaccharides > corn syrup solids > maltodextrins > starches. The amino acid present is also dependent upon the reaction rate (glycine is the most reactive) [23].

As mentioned previously, the water activity is another parameter that influences Maillard reaction. The water activity affects the rate and pathway of Maillard reaction. While some reactions produce water as a by-product (reaction pathway is inhibited by water), other chemical reactions consume or require water (reaction pathway is promoted by water). There are numerous chemical reactions that occur and may be accelerated at low water activity. A maximum browning reaction occurs in most foods between water activities 0.3 and 0.7 [24]. For example, in sugar-amino browning reaction in a food system at higher water activities, the decrease in reaction rate has been generally attributed to dilution of the reaction partners. The decreased reaction rate at low water activities, when the amount of mobile water is lowered, has been ascribed to an increasing diffusion resistance which lowers the mobility of the reaction partners [24]. These results are in conformity with data from model systems for Maillard reactions where browning is strongly increased at low water contents [11, 25]

The pH of the system is another parameter that influences Maillard reaction, affecting the rate and pathways. In some cases, the pH can be used as an indication for the step to which the Maillard reaction has occurred. As organic acids are formed along with the reaction and as their concentration increases, the buffer concentration becomes insufficient to buffer the system, resulting in a pH drop [20].

9.3.2 Flavor from Lipids

The different lipids may undergo changes during the processing of foods and therefore can contribute to the flavor. Between all food ingredients, lipids have an important organoleptic impact, probably the strongest. Reactions such as lipid oxidation and lipolysis can occur, giving undesirable flavors at the same time. The combinations of these reactions are necessary for the evolution of the character-impact aroma compounds in different foods [26]. Foods such as French fried potatoes, hamburgers, snacks and cheese are generally well-liked by consumers. While their popularity may in some measure lie in the physical properties secured to the food by fats (lubricity, crispness, richness, and texture) [27]. This flavor comes from both thermally-induced changes in the food (Maillard reaction) and flavor developed in, e.g., the frying oil. Most of the flavor compounds in fats and oils are produced by the reaction of oxygen with unsaturated fatty acids from triacylglycerols or polar lipids. The flavor compounds development by oxidation of lipids have considerable negative effects on consumers in processed food and edible oils [27, 28, 29].

An example of which lipids affect the food flavor can be seen, although diverse meats and products show different effects of high pressure on their oxidative stability, depending on pressure level, temperature and duration. It has long been known that heat treatment of meat initiates lipid oxidation and enhances the development of off-flavors during long-term storage. At the same time, before heating the flavor of meat is transformed and new compounds appear, mainly by lipid oxidation and Maillard browning reactions, which are mutually responsible for the desired cooked flavor of meat.

The oxidative deterioration is of greatest economic importance in the production of lipid-containing foods. Oxidation of unsaturated lipids not only produces offensive odors and flavors, but can also reduce the nutritional value and safety by the formation of secondary reaction products in foods after cooking and processing [27, 30].

9.3.2.1 Autoxidation

Autoxidation is the reaction of unsaturated lipids with oxygen to form hydroperoxides. This oxidation is in most instances a free

radical chain reaction involving three basic steps of initiation, propagation, and termination.

Initiation:

$$RH \xrightarrow{\text{Light, heat or metal}} R\bullet + H\bullet$$

$$ROOH + M^{n+} \longrightarrow RO\bullet + M^{(n+1)+} + OH$$

$$ROOH + M^{(n+1)+} \longrightarrow ROO\bullet + M^{n+} + H^{+}$$

$$2ROOH \longrightarrow ROO\bullet + RO\bullet + H_2O$$

The production of free radicals may take place by direct thermal dissociation (thermolysis), by hydroperoxide decomposition, by metal catalysis and by exposure to light (photolysis) with or without the intervention of photosensitizers.

In a second step, the propagation, propensity of organic substrates to autoxidation depends on their relative ease to donate hydrogen by reaction. With unsaturated fats, susceptibility to autoxidation is dependent on the availability of aIIylic hydrogens for reaction with peroxy radicals. The lipid-free radical, therefore produced, can additionally react with oxygen to produce a peroxy radical which then produces a chain reaction. Reaction rate between oxygen and alkyl radical is fast, for this reason the majority of the free radicals are in the form of peroxy radical.

Propagation:

$$R\bullet + O_2 \longrightarrow ROO\bullet$$

$$ROO\bullet + RH \xrightarrow{\text{Slow}} ROOH$$

$$RO\bullet + RH \longrightarrow ROH + R\bullet$$

Finally, in an autoxidation reaction, the termination step takes place. Consequently, the main terminations are via the interaction between two peroxy radicals, as can be seen below.

Termination:

$$R\bullet + R\bullet \longrightarrow R\text{-}R$$

$$R\bullet + ROO\,R\bullet \longrightarrow ROOR$$

$$ROO\bullet + ROO\bullet \longrightarrow ROOR + O_2$$

The mechanism of flavor formation in heated oils is fundamentally that of lipid oxidation. The thermally-induced oxidation involves hydrogen radical removal, the addition of molecular oxygen to form the peroxide radical, formation of the hydroperoxide and next decomposition to form volatile flavor compounds. The lipids oxidation products formed at room temperature differ from the products of thermally-induced oxidation. As in the Maillard reaction, these differences go back to kinetic considerations. Consequently, the reactions taking place in the frying oil and volatiles formed are, thus, related with the temperature used.

The effects of irradiation on lipid oxidation and off-flavor generation in cooked meat might be different from those in raw meat [30]. Ramarathnam *et al*. reported that in cooked meat, hexanal was the major lipid oxidation-related volatile, but the contribution of other aldehydes such as heptanal, octanal, and nonanal to the off-flavor of cooked meat was also significant because of their high flavor dilution factors [31, 32]. 1-Heptene and 1-nonene increased proportionally with the increase of irradiation doses. The packaging also influences the flavor from lipids; storage in aerobic packaging increased the amount of 1-heptene and 1-nonene because of lipid oxidation. Other volatile compounds such as aldehydes, ketones, and alcohols were not influenced by irradiation in a first moment (day 0), but irradiation accelerated lipid oxidation and increased the amount of those compounds after storage [32].

Lipid oxidation products may also occur during procedures such as roasting, e.g., during roasting of nuts and seeds. These are much appreciated worldwide as snack foods and appetizers. The thermal treatment helps to improve flavor, food microbiological safety, and shelf life in food. Furthermore, during roasting the production of potentially beneficial compounds are promoted, with antioxidant properties related to the formation of phenol-type structures and/or the chelating properties of melanoidins. However, the roasting process can also induce the development of undesirable reactions that may originate loss of nutritional value, as well as formation of potentially toxic compounds. The major oxidative reaction in heat-treated nuts and seeds (as well as in other lipid-rich foods) is lipid peroxidation. Lipid oxidation in foods is essentially autocatalytic and involves, almost exclusively, unsaturated fatty acids which increase reaction rates with time. Seeds, nuts and dried fruit contain high amounts of unsaturated fatty acids, making them very susceptible to rancidity. Strong heat treatment of lipid-rich foods

may also induce the formation of trans fatty acids (by isomerization of double bonds). The secondary lipid oxidation products, namely, the aldehydic compounds formed during peroxidation, are prone to formation of lipoxidation end products during the Maillard reaction by nucleophilic attack of the carbonyl compound on the free amino groups of amino acids or proteins.

9.3.2.2 Hydroperoxides

Another reaction that can occur during processing or preparation of a food with lipids is the formation of hydroperoxides. In this case, free radical mechanism of hydroperoxide formation involves the abstraction of hydrogen atom from the α-methylene group of a lipid molecule [33].

For example, for oleate, the hydrogen abstraction on C-8 and C-11 generates two allylic radicals. In a next step, the intermediates react with oxygen to produce a mixture of 8-, 9-, 10- and 11-allylic hydroperoxides. Autoxidation of linoleate involves hydrogen abstraction on the doubly reactive allylic C-11, with the formation of a pentadienyl radical. This intermediate radical reacts with oxygen to produce a mixture of conjugated 9- and 13-diene hydroperoxides. In the case of two separate 1,4-diene systems, such as linolenate, hydrogen abstraction will occur on the two methylene groups, C-11 and C-14. The intermediate free radicals react with oxygen to form conjugated dienes with hydroperoxides on C-9 and C-13, or C-12 and C-16, while the third double bond remains unaffected [30].

As a result of the instability of hydroperoxide products, unsaturated fatty acids formed by autoxidation are broken down into a broad variety of volatile flavor compounds (as well as non-volatile products). The decomposition of hydroperoxide involve homolytic cleavage of the -OOH group. It is widely accepted that hydroperoxide decomposition involves homolytic cleavage of the -OOH group, proving radicals such as alkoxy and hydroxyl [33].

9.3.2.3 Lipoxygenase Pathway

The lipoxygenase pathway is accepted for the production of volatile compounds of six and nine carbons, among others. Most of the alcohols, aldehydes, and esthers that contribute to fruit and vegetable aroma are generated for oxidative degradation of linoleic and linolenic acid [34]. Lipoxygenases are a family of dioxygenases that catalyze the stereospecific insertion of molecular oxygen into

unsaturated fatty acids containing at least one 1,4-cis, cis-pentadiene unit to form fatty acid hydroperoxides. Many important flavor compounds in plants result from the enzymatic degradation of unsaturated fatty acids [35]. These compounds are the principal substrate of lipoxygenase pathway. During lipoxygenase pathway can also develop off-flavors in foods of plant and animal origin [36]. The lipoxygenase pathway is involved in the production of flavor compounds as well as in plant defense, plant development and plant communication. These aromas include both volatile alcohols and carbonyls which are derived from polyunsaturated fatty acids through lipoxygenase-mediated reactions.

9.3.3 Flavors Formed via Fermentation

During the fermentation process important compounds are developed which fundamentally modify the flavor of foods. Fermentation is used in a wide variety of foods from different sources. Some examples include wine, beer, caper, cheese, yogurt, soy sauce, bread, olive, and fermented fish products [37, 38]. The fermentation of sugars by yeast is an old and well known technology where carbohydrates are transformed into different compounds such as water, ethanol, carbon dioxide, etc. In other cases, the flavor of these products may be developed from residual enzymatic activity once the microbial cell has lysed [39]. Lactic acid bacteria are other typical examples of the importance of fermentations. These are essential agents during meat fermentation, improving hygienic and sensory quality of the final product. Its fermentative metabolism prevents the development of spoilage and pathogenic microflora by acidification of the product, also contributing to its color stabilization and texture improvement. Lactic acid fermentation of vegetables nowadays has an industrial significance for cucumbers, cabbages and olives. Several other varieties of vegetables (e.g., carrots, artichokes, French beans, marrows, capers and eggplants) also increase their safety, nutritional, sensory and shelf-life properties through lactic acid fermentation under standardized industrial conditions. The fermentation process is also involved in the flavor of bread [40]. In general, the flavor of bread has been acknowledged to be influenced by choice of ingredients, enzymatic reactions occurring during dough fermentation by yeasts and/or lactic acid bacteria, and thermal reactions induced during baking [37, 39, 41].

The fermentation may be allowed to proceed spontaneously, or can be "started" by inoculation with a must that has been previously successfully fermented by desired microoorganism. Actually, in most fermentation processes the original microbial population is removed with pasteurization or by treatment with sulphur dioxide, and later is inoculated with a starter culture derived from a pure culture. This procedure eliminates many of the uncertainties and difficulties of older methods. Following is a description of some metabolites that bring different flavors to fermented foods.

9.3.3.1 Acids

The flavor of numerous fermented foods is due to acid production via microorganisms. The lactic acid is, perhaps, the acid of greatest importance to the flavor in fermented dairy products (yogurt, cheese), table olive, capers, pickles, etc. Lactic acid bacteria are used as (mixed) started cultures for the production of fermented foods. The sensory properties of a product are modified during the fermentation process and lactic acid bacteria influence the flavor development. Flavor compounds are formed by the conversion of lactose and citrate (glycolysis and pyruvate metabolism), by lipolysis and by proteolysis and conversion of free amino acids. Composition of started culture can influence final product flavor. Moreover, flavor formation in fermented food products largely depends on the substrate. Differences in the presence of flavor compound precursors may result in different fluxes through flavor pathways

The organisms most commonly associated with lactic acid formation are classified as being either homofermentative (e.g., *Lactobacillus bulgaricus, L. plantarum, L. acidophilus*) or heterofermentative (e.g., *Leuconostoc sp., L. brevis, L. fermenti*) which produce lactic acid, acetic acid, ethanol, carbon dioxide, and other metabolites. Lactic acid is also present in wines because during malolactic fermentation malic acid is transformed into lactic acid. This malolactic fermentation is the result of bacterial (most commonly *Leuconostoc sp.*) action during the last stages of fermentation.

Several organic and aliphatic acids are produced during fermentation. Other pathways to production of acid in foods are lipase systems present in most microorganisms and deamination of amino acids. These enzymes attack triglycerides to yield glycerol, monoglycerides, diglycerides, and free fatty acids. The flavor of various foods, such as cheese and cured meats, can be considerably

affected by lipase systems action. The conversion of glutamine into glutamate during bread fermentation is an example of deamination. The accumulation of glutamate rather than glutamine in sourdough may be attributable to glutamine deamidation by lactobacilli. High glutamate levels are related to the savory taste of foods and the accumulation of glutamate in sourdoughs may contribute to bread flavor [40, 42, 43].

9.3.3.2 Alcohols

Alcohol and acids are two primary products of fermentation, both used with good effects in the preservation of foods. Several alcohol-fermented foods are preceded by an acid fermentation and in the presence of oxygen and acetobacter, alcohol can be fermented to produce acetic acid. Most food spoilage organisms cannot survive in either alcoholic or acidic environments. Therefore, the production of both of these end products can prevent a food from undergoing spoilage and extend its shelf life. The foods and beverages included are wines, beers, Indonesian tape ketan/tape ketella, Chinese lao-chao, South African kaffir/sorghum beer and Mexican pulque. These are generally yeast fermentations but they also involve yeast-like molds such as *Amylomyces rouxii* and mold-like yeasts such as *Endomycopsis* and occasionally bacteria such as *Zymomonas mobilis*. The substrates include diluted fruit juices, sugarcane juice, honey, palm sap, germinated cereal grains or hydrolyzed starch, all of which contain fermentable sugars that are quickly converted to ethanol in natural fermentations by yeasts in the environment [44].

The flavor of yogurt alcohol is also present, and is another group of volatile compounds found in yogurt. The principal alcohol in yogurt is ethanol, which is a common terminal end product in the breakdown of glucose and catabolism of amino acids. Alcohol production from amino acids may occur either by transamination, decarboxylation, and reduction, or by oxidative deamination followed by decarboxylation and reduction. In either pathway, the product is always the amino acid minus the amine group and one carbon atom [45].

9.3.3.3 Carbonyls

Diacetyl is an important carbonyl contributor to the flavor of fermented dairy products. It is characterized by having a buttery,

nut-like aroma, which is probably the major flavor component; although a number of other flavors are present in these types of foods. Diacetyl is produced via citrate fermentation; citrate is present naturally in cow's milk [39]. At pH 5.0–5.2 lactic acid displaces citric acid from its salts; in the presence of lactose the free citric acid is converted into diacetyl. The biochemical pathway can be summarized as follows: citrate → oxalacetate → pyruvate → acetolactate → diacetyl [46]. The most known citric acid fermenters are *Leuconostoc citrovorum*, *L. creamoris*, *L. dextranicum*, *Streptococcus lactis subspecies diacetylactis*, and *S. Thermophilus*. In alcoholic beverages (principally in wine and beer) at low levels, diacetyl contributes a slipperiness to the feel in the mouth.

It should be noted that diacetyl is a unstable compound. The yeast then absorbs the diacetyl and reduces the ketone groups to form acetoin and 2,3-butanediol, relatively flavorless compounds. During beer elaboration sometimes it is desirable for a small concentration of diacetyl to remain. For this reason, the temperature is raised for two or three days to allow the yeast to absorb the diacetyl produced earlier in the fermentation cycle [47].

The volatile flavor compounds in cheese originate from degradation of the major milk constituents (i.e., lactose, citrate, milk lipids, and milk proteins) during ripening, which, depending on the variety, can be a few weeks to more than two years long. Carbonyl content and methyl ketone composition of cheese (especially blue cheese) have been studied at progressive stages of ripening. Carbonyl compounds are produced from the milk fatty acids by the metabolic activity of the mold *P. rogueforti*. The milk fat is hydrolyzed by the lipases of the mold and the free fatty acids are subsequently metabolized into methyl ketones and other carbonyl compounds. Generally, dairy products contain a significant quantity of α-keto acids which are readily hydrolyzed from the triglyceride by microbial lipases and then decarboxylated to form odd carbon number methyl ketones.

As was mentioned in the section about lipoxygenase pathway, methyl ketones and aldehydes may also be formed via microbial-induced lipid oxidations. The hydrolysis of triglycerides (about 98% of cheese fat) is the principal biochemical transformation of fat during ripening, which leads to the production of free fatty acids, di- and monoglycerides and possibly glycerol. Free fatty acids contribute to the aroma of cheese. Individual free fatty acids, particularly acids between C4:0 and C12:0, have specific flavors (rancid,

sharp, goaty, soapy, coconut-like). The flavor intensity of free fatty acids depends not only on the concentration, but also on the distribution between aqueous and fat phases, the pH of the medium, the presence of certain cations (that is, Na^+, Ca^{2+}) and protein degradation products [48, 49].

Also mentioned is another pathway of flavor compounds via fermentation, which refers to transamination and decarboxylation of free amino acids. Cheddar cheeses with unclean-type flavors that were described as subtle "floral" or "rose-like" aftertastes were mostly aged. This flavor note is attributed to compounds like phenyl ethanol and phenyl acetaldehyde, which are originated from the hydrophobic amino acid, phenylalanine, through enzyme-mediated transamination, decarboxylation, and reduction reactions [48, 50].

9.3.3.4 Terpenes

Terpenes are ubiquitous throughout the food chain; therefore, it is not surprising that they serve as effective flavoring ingredients. They are often used to convey citrus, pine, balsamic, woody and fruity notes. Terpenes are hydrocarbons based upon the five carbon isoprene unit (2-methyl-1,3-butadiene) with structures that may be open chain, closed chain, saturated, or unsaturated, and may contain O, N, or S. Terpenes are principally formed by microorganisms, however, no fermented, commercially available food products derive their characteristic flavor from terpenes [51, 52]. Herbs and higher plants containing terpenoids and their oxygenated derivatives have been used as fragrances and flavors for centuries [52].

Aroma volatiles in the juice of mandarin contain terpenes such as D-limonene, myrcene, sabinene, α-pinene and linalool. Furthermore, it was suggested that some of these terpenes could be used as quality control parameters in mandarin juices, since contents of α-terpineol and terpinen-4-ol increased in processed juices and their accumulation was negatively correlated with juice acceptability. The oxidation of terpenes, such as limonene, imparts lower quality to citrus juice. They is also an indication of peel oil components, richer in terpene compounds and usually higher in commercially processed juice [53].

9.3.3.5 Esters

Esters are responsible for the fruity character of fermented beverages, and volatile esters constitute an important group of

aromatic compounds in alcoholic beverages such as beer and wine. The biochemical formation of yeast-derived, sensory-active metabolites like higher alcohols and esters determines the different characteristics of aroma and taste in fermented beverages. In these beverages, volatile esters are only trace compounds, but they are extremely important for the flavor profile of these drinks. Even small changes in the concentrations of these secondary metabolites can have large effects on the final sensorial quality of fermented beverages. Esters are produced intracellularly by fermenting yeast cells and are of major industrial interest for their contribution to the flavor in fermented beverages [47, 54, 55]. Ethyl esters can diffuse through the cellular membrane into the fermenting medium due to their lipid solubility. On the contrary, acetate ester excretion is rapid and complete and the transfer of ethyl esters to the fermenting medium is severely reduced with increasing chain length, from 100% for ethyl hexanoate to 54 to 68% for ethyl octanoate and 8 to 17% for ethyl decanoate. The rate of ethyl ester formation is dependent on different factors: concentrations of the two co-substrates (the acyl coenzyme A component and ethanol) and the activity of enzymes involved in their synthesis and hydrolysis [56].

In fermented beverages there are two central groups of flavor-active esters. Acetate esters (in which the acid group is acetate and the alcohol group is ethanol or a complex alcohol derived from amino acid metabolism), such as ethyl acetate (solvent-like aroma), isoamyl acetate (banana aroma), and phenyl ethyl acetate (roses, honey) are the first group of flavor-active esters. The second group is the ethyl esters (in which the alcohol group is ethanol and the acid group is a medium-chain fatty acid) and includes ethyl hexanoate (anise seed, apple-like aroma), ethyl octanoate (sour apple aroma), and ethyl decanoate (floral odor). The acetate esters have received the most attention of the two groups, not because they are more important but because they are produced at much higher levels and therefore are easier to measure. Contrarily, much less is known about ethyl ester production, despite the desirable apple-like aromas of ethyl esters [47].

The esters are also formed by esterification of various acids and alcohols during processing of ham. This chemical family strongly affects the flavor of ham as a typical aged meat product; in particular, the methyl-branched short-chain esters were found to be positively related to the attribute of aged meat [57]. Lactic acid bacteria have been reported in processes of amino acid deaminases

and transaminases, which can convert flavorless amino acids into aroma-bearing short/branched-chain keto acids and esters precursors [58].

9.3.3.6 Sulphur Compounds

The organic sulphur compounds have a considerable influence on the sensorial quality of many foods and beverages, such as wine, ham, fermented dairy products (cheese, cream). For example, in wines they usually give off unpleasant odors, although some of them may contribute to the desirable aroma [59]. In a study about dry-cured ham it was observed that sulfide compounds were formed principally from the sulfur-containing amino acids, such as methionine, cysteine and cystine, via Strecker degradation to thiols. Dimethyl disulfide was an oxidation product of methane thiol, and could react to form dimethyl trisulfide and dimethyl sulfide. In this study, aldehydes, ketones and sulphur compounds that were found at post-aging were directly derived from amino acids [57].

In wine, for example, organic sulphur compounds can be classified into two groups: those with a boiling point lower than 90ºC (volatile compounds) and those with a boiling point above 90ºC (heavy compounds). Due to their low sensory thresholds, the volatile compounds contribute significantly to the possible disagreeable flavor of wine. The aroma of wine can be critically affected by low-volatility sulphur compounds either because of the concentration at which they are present or because they become precursors of volatile sulphur compounds, which are degraded throughout the elaboration and storage of wine. In any case, the sulphur compounds are normally found at trace levels in wines [60].

During cheese ripening, the microbial and enzymatic systems produce volatile sulphur compounds, proving real technological promise for this research area [58]. The production of sulphur flavor by fermentation in milk and cream reported the 3-methylsulfanylpropan-1-ol (methionol) as the predominant volatile sulphur flavor compound together with dimethyl disulphide, S-methyl thioacetate, 3-methylthio-1-propanoic acid, methional, 3-methylthio-1-propene, and ethyl 3-methylthio-1-propanoate [61]. The methionol sulphur flavor in cream is concentration-dependent, ranging from a savory-slight potato note to a strong potato-savory note to a cooked cheese potato note. Other examples of sulphur flavor are methional in cheese which has a boiled potato-like

aroma and is an important contributor of good Cheddar aroma and methanethiol, dimethyldisulphide and dimethyltrisulphide, which likely contribute to the desirable garlic note of cheeses [62].

It is important to note that sulphur dioxide is also used in vinification or beer elaboration as an antiseptic against undesirable microorganisms and as an antioxidant against the effects of oxygen. In this case the sulfur dioxide is not synthesized, but is added during these processes by control fermentation [60].

9.4 Special Industrial Process and Flavor

As mentioned above, there are several chemical reactions that contribute different flavors. Several of these reactions are dependent on temperature, water activity, among others.

Fresh milk has an agreeable, slightly sweet flavor, with small aroma and a pleasant mouth feel and aftertaste. Some changes to these characteristics is considered a defect. The milk flavor is affected by numerous factors, including the physical condition of the cow; the feed consumed by the cow; biological and enzymatic changes in milk; etc. The heating regimes used to destroy pathogenic bacteria and inactivating enzymes deeply affects the milk flavor. At ultra-high temperature (UHT), the treatment most widely used is the sterilization technique; milk is heated at temperatures higher than 130ºC (138–145ºC) for a holding time of 1–10 s, followed by aseptic packaging.

In milk, raw and processed, several volatile sulphur compounds have been detected. Many of these compounds are related to the cooked flavor and increase during heat processing, especially UHT and sterilized milk. Much research has been carried out trying to explain the origin of sulphur compounds in both raw and processed milk, and how to diminish the associated cooked flavor that has a negative impact on consumer acceptability of processed milk. This cooked flavor which develops during UHT process could disappear after a few days of storage. The effect of volatile sulphur compounds on flavor varies among dairy products. While they are responsible for off-flavor in UHT milk (cooked flavor), they also contribute to the typical flavor of Cheddar, butter, etc. [63, 64]. Upon further storage, other flavors are developed such as heated, oxidized, or stale. In addition, rancid flavors due to lipolysis, and bitter flavor due to proteolysis may also develop. One of

the frequent problems of the dairy industry is the perceived poor flavor of UHT milk, which has become an important barrier to consumer acceptance of UHT milk.

Another industrial and conservation process that affects food flavor is irradiation. Food irradiation is an economically viable technology for the reduction of postharvest losses, extension of shelf life of perishable commodities, improvement of hygienic quality of foods and elimination of pathogenic bacteria (such as Salmonella, Listeria monocytogenes and Escherischia coli O157:H7) from different foods.

Irradiation is the best-known intervention strategy that can ensure the safety of raw meat. However, even at low doses, irradiating fresh meat can result in off-flavors which have been described as bloody, rotten egg, fishy, burnt, barbecued corn, sulfur, metallic, alcohol or acetic acid. In this procedure, parameters, such as type of meat, temperature during irradiation, oxygen exposure during and/or after the irradiation process, packaging and presence of antioxidative substances, influence the flavor. Increasing the irradiation dose increases these compounds, however, cooking reduces them. A broad range of flavor- and odor-active volatiles is present in meat, such as acids, alcohols, aldehydes, aromatic compounds, esters, ethers, furans, hydrocarbons, ketones, lactones, pyrazines, pyridines, pyrroles, sulfides, thiazoles, thiophenes, pyrroles, and oxazoles [65, 66].

The irradiation-induced reactions are not the result of a statistical distribution of random cleavage of chemical bonds. The progress of radiolysis follows preferred pathways which are largely influenced by molecular structure. The primary events involve the formation of excited molecules and molecule-ions [67]. In foods that are irradiated more than 7% of the volatiles found are hydrocarbons commonly present in thermally-processed and unprocessed foods [68]. The changes in irradiated meat are related principally with lipid oxidation, free radical reactions and/or to generation of volatile sulfur compounds.

Fresh spinach is another example of food irradiation. Components such as oxygen radical absorbance capacity and total phenolic content were not significantly affected by irradiation, while the ascorbic acid content decreased. However, the ascorbic acid content of irradiated sample decreased rapidly during storage; nevertheless, sensory evaluation (appearance, aroma, texture, flavor, etc.) were not affected by irradiation [69]. Fresh ginger rhizomes, carrot and

cucumber also were irradiated and differences could be noted in qualitative and quantitative volatile aroma constituents. It was concluded that gamma irradiation avoids sprouting and increases the shelf-life of fresh vegetables under ambient conditions, without affecting its flavoring principles [70, 71]. Finally, irradiation can produce a characteristic aroma, accelerate lipid oxidation and change the color in food, besides prolonging its useful life.

Ohmic heating is a thermal processing method wherein the food material is heated by passing electricity through it, which serves as an electrical resistor. Electrical energy is dissipated into heat, which results in rapid and uniform heating. Ohmic heating is also called electrical resistance heating, Joule heating, or electro-heating, may be used for a variety of applications in the food industry. In conventional thermal processing, the product quality may be damaged due to conduction and convection heat. During ohmic heating the entire mass of food is volumetrically heated, so the quality of product is far greater than in a food which is conventionally heated. For example, food such as liquid egg can be ohmically heated rapidly without coagulating it. Another example is fruit juice, which can be treated to inactivate enzymes without affecting the flavor. Different heating processes that have been tested on orange juice found that the usual pasteurization treatment can negatively affect the flavor. The ohmic-heated juice had higher aroma volatile concentrations than conventional pasteurization [13, 72].

Another "special industrial process" that influences food flavor is extrusion. Extrusion is a procedure whereby foods of low-moisture content (around 10–30%) are submitted to the action of pressure, heat, and mechanical shearing for a small amount of time. Extrusion processing is applied in different industries such as food and pharmaceutical industries, for affecting product microstructure, product chemistry or the macroscopic shape of products. Consequently, it can have important effects on the flavor of food products. Extrusion is a significant industrial process in the manufacture of pasta, ready-to-eat cereals, snacks, pet foods, and textured vegetable protein. With respect to temperature, this process can be applied at high temperatures, known as cooking extrusion. In the manufacture of ice cream it also has been developed in sub-zero-ºC processes. Extrusion cooking technology is an efficient and versatile method to change raw materials into final food products and has been used to develop various types of snack foods, mainly from corn meal, rice, wheat flour or potato flour, in many

shapes and a variety of textures. The effects of extrusion cooking on nutritional quality are ambiguous. The extrusion process inactivates some antinutritional factors, denatures undesirable enzymes, sterilizes the finish product and retains properties as natural colors [73, 74, 75]. The sensory characteristics of extruded snacks are critical for consumer acceptance. These could include crisp and well-expanded texture, homogeneous structure, good taste, attractive appearance, color and aroma. On the other hand, certain mechanisms such as lipid oxidation and nonenzymatic browning are considered to have significant implications in the flavor characteristics of food products. During this industrial process, this reaction frequently does not take place thereby affecting the flavor; for example, when the moisture content is low, lipid degradation decreased and Maillard reaction products dominated the flavor profile. However the lipid oxidation occurred during storage of these foods [76]. Some research has indicated that the aroma in extruded foods could be low, possibly due to the high temperature used in cooking extrusion. The color also changes during this process. Change of this important quality characteristic of extruded foods may be present due to decomposition of pigments; product expansion causing color fading; or the color produced as a result of chemical reactions, as mentioned above [75].

9.5 Industrial Production of Flavor

Initially the flavor industry put great effort into attempting to duplicate nature through the use of synthetic chemicals. In the same way, formulation methods are becoming increasingly important in the product design of flavors. The perception of food flavor is the result of a multitude of interactions between a large number of chemical compounds and sensory receptors. Compounds interact, combine and show synergistic (i.e., the presence of one compound enhances the perception of another) and antagonistic (a compound suppresses the perception of another) interactions. These large numbers of chemicals make them very difficult to obtain artificially. Also, frequently flavorings isolated from nature have not performed well due to detrimental components original to the flavoring. For these reasons, the main activities of flavor industries are collection or production of flavoring materials, manufacturing flavor, studies on flavor application, and technical services. However,

in some cases, due to the low abundance of the volatile compounds in plant sources, the natural products had been replaced by synthetic analogues.

Roughly, there are two methods to extract flavor compounds, distillation and solvent extraction. During distillation, a complex mixture of organic compounds is obtained, namely essential oil. The essential oil is responsible for the characteristic flavor of the plant material. In citrus peels, for example, the essential oils can be extracted to cold-pressed [77]. When flavor compounds are nonvolatile a solvent can be used to remove them. Essential oils do not contain water soluble flavoring components, antioxidants or pigments [78].

In addition, consumers demand high standards of safety, quality and nutrition in ready-to-eat food. Because of this, continuous advancement in conventional processing technologies and the development of new alternatives are needed. Flavors represent important challenges in terms of process engineering because they cover a very broad range of physical and sensory characteristics, are on occasion unstable and are perceived by human beings on the basis of very complex, extremely nonlinear mechanisms. Thus, the food industry constantly looks for ways to preserve the food flavor. Several aroma compounds employed to flavor food products are used in a solid state, after encapsulation. The encapsulation of flavor ingredients is very attractive and widely investigated in food science. Complexation of aroma can improve food flavoring by reduction of evaporation, and control their release during storage and application. This is a procedure in which small particles or droplets are enclosed by a coating to give small capsules with many useful properties. Different polymers (synthetic or natural) are the common matrices used to entrap these flavors. The system should be prepared from food-grade ingredients utilizing reliable and economical processing operations. When employed, encapsulation allows distribution of liquid flavors in standardized and functional forms, providing numerous benefits. The encapsulation is also used to protect various food components.

Flavor encapsulation methods are numerous, for example, spray drying, melt extrusion, fluidized bed coating, complex coacervation, and liposome entrapped coating, etc. All of these methods have both advantages and disadvantages, so the choice of the correct process is significant to a successful release of the encapsulated flavor [79].

The method of spray drying has been used many times to encapsulate various kinds of food ingredients. Spray drying is frequently used to dry foods and ingredients that are sensitive to heat, for example flavor oils, proteins, and lipid droplets. A characteristic of this methodology is that the powders obtained are easy to reconstitute. In this method a suspension or liquid solution is passed through a small nozzle, which produces a mist of fine drops. The outlet of the nozzle is located in controlled high temperature environment so that the volatile liquid phase (frequently water) within the drops quickly evaporates. Spray drying is able to produce a dry powdered product in continuous operation [80].

During a melt extrusion encapsulation process, a carbohydrate is mixed with flavor compounds and melted by a combination of heat and shears in the extruder barrel, and finally pressed through orifices (extrusion). So, the crystalline structure is converted in an amorphous phase. This amorphous phase contains active agent with relatively small mobility. Microcapsules made via extrusion may show stickiness, clumping and structural imperfections. The structural imperfections could limit their shelf life [79].

The fluidized bed coating is another mechanical encapsulation process in which a coating is applied onto power particles. This process can be in batch or continuous mode. During this method, solid particles to be encapsulated are suspended on a current of air and then coated by a spray of liquid coating material. After, their shells are solidified by cooling or solvent vaporization. The procedure of suspending, spraying and cooling can be repeated until that shell capsulate is the one desired [80].

Complex coacervation is a chemical method by obtained capsules. This method is made via a liquid-liquid phase separation mechanism of an aqueous solution into two phases, a polymer rich phase and a polymer poor phase. The polymer rich phase is called complex coacervate. This generates a three immiscible phase system (oil, polymer rich, and polymer poor phase). So, the polymer rich phase droplets will deposit on the emulsion surfaces by interfacial sorption. The process can be simple when only one type of polymer is used or complex coacervation when two or more types of polymers of contrary ionic charges are present [81].

Liposome entrapment is other method employed to obtain capsules. This consists of at least one closed vesicle composed of bilayer membranes made with lipid molecules, such as phospholipids and

cholesterol. The vesicles are formed dispersing the lipid in aqueous media and shear rate by microfluidization or colloid mill [79].

9.6 Summary

Humans are capable of recognizing five main taste qualities: sour, sweet, bitter, salty and savory (umami), and maybe several subqualities. This number of tastes is comparatively small if compared with the number of chemical compounds that elicit taste. In the last years, with the advent of sophistical instruments to separate and measure aroma compounds, the researchers have an increasing knowledge about the flavor and aroma compounds, and their interaction and behavior in different foods. Even so, there is much to learn about flavor. The new technological processes used in food elaboration are other important topics in food flavor, making it a dynamic subject matter. The multitude of interactions between all components and environmental factors (such as temperature, water content, etc.) give the final sensorial quality to a food and beverage.

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10

New Trends in Sensory Characterization of Food Products

Gastón Ares* and Ana Giménez

Department of Food Science and Technology, Faculty of Chemistry, Universidad de la República, Montevideo, Uruguay

Abstract

Sensory characterization provides a complete description of the sensory characteristics of food products. Descriptive Analysis is the most common methodology for this purpose. However, due to the cost and time associated with its application, several alternative methods have been recently developed. These methods do not require training, can be performed by trained assessors or consumers, and have been reported to be a good option when quick information about the sensory characteristics of a set of products is needed. There are basically four types of methodologies: methodologies based on the evaluation of specific attributes, on global differences among products, on the comparison with references, and methodologies that provide a verbal description of the products. In the present chapter these novel methodologies for sensory characterization of food products are described. Advantages and disadvantages of each methodology are discussed and recommendations for their application are provided.

Keywords: Descriptive Analysis, consumer profiling, projective mapping, CATA, sorting, polarized sensory profiling

10.1 Introduction

10.1.1 Sensory Characterization

Sensory characterization is one of the most powerful, sophisticated and extensively applied tools in sensory science, which aims

**Corresponding author*: gares@fq.edu.uy

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at providing a complete description of the sensory characteristics of food products [1]. Sensory characterization is extremely useful for the food industry when detailed information about the sensory characteristics of food products is needed. In particular, this methodology is usually applied for the development and marketing of new products, the reformulation of existing products, the optimization of manufacturing processes, monitoring the sensory characteristics of the products available in the market, the implementation of sensory quality assurance programs, establishing relationships between sensory and instrumental methods, and for sensory shelf life estimation [2].

One of the most common applications of sensory characterization is during new product development and product reformulation. At these stages it is usual that product formulation and processing conditions are systematically varied following an experimental design in order to generate a series of prototype products [3]. In this context, sensory characterization enables the product developer to evaluate how formulation and processing variables affect the sensory characteristics of the prototypes, to determine how close the prototypes are from the target product to be developed and to take decisions based on objective sensory information.

Furthermore, during the implementation of sensory quality assurance programs sensory characterization plays a key role in defining specifications or quality standards for the sensory characteristics of food products, as well as for establishing specifications for physicochemical properties that are related to specific sensory characteristics [4].

10.1.2 Descriptive Analysis

Several classical methodologies have been used for sensory characterization of a wide range of food products, which are known as Descriptive Analysis techniques. These include the Flavour Profile® [5, 6], Texture Profile® [7, 8], Quantitative Descriptive Analysis® (QDA) [9, 10], and Sensory Spectrum® methodologies [11, 12].

The most commonly used methodology, usually known as Descriptive Analysis, is an adaptation and combination of the basic features of traditional QDA and Sensory Spectrum [1]. Descriptive Analysis should be performed with a panel of 8-20 trained assessors,

and involves three basic steps: (i) descriptor generation, (ii) assessor training, (iii) evaluation of samples.

The first step of Descriptive Analysis involves the selection of the main attributes that characterize the product's sensory properties, by generating a complete list of descriptors. This step is performed by asking the assessors to select the descriptors that describe the sensory characteristics of a wide range of products within the specific product category; this can be done by consensus or by providing a list of all the possible words that describe the category [1]. Once the descriptors are selected, the evaluation technique should be clearly defined and references should be selected to help the assessors to identify and quantify each sensory attribute [13].

After descriptor generation the assessors should be trained in attribute recognition and quantification since quantitative information is a key point in Descriptive Analysis [10]. Usually, attribute intensity is quantified using a 10 cm or 15 cm line with words such as slight and intense at the extremes. During successive sessions the assessors are presented with different samples and are asked to quantify the intensity of each of the selected attributes. The length of the training process usually ranges from 10 h to 120 h, depending on the complexity of the specific product and the number and characteristics of the sensory attributes needed to characterize the product [2, 14]. In order to define the end of training, i.e., when the panel is capable of providing reliable information about the sensory characteristics of food products, the performance of each assessor, as well as the performance of the entire trained panel, should be checked [15]. Panel performance is evaluated considering repeatability, reproducibility and discrimination [16]. After training, all the assessors should be able to score the same product consistently for a given attribute to find differences between products and to evaluate them, on average, as the rest of the trained panel [17]. Checking panel performance is usually carried out using analysis of variance and multivariate statistical techniques [18–21].

An advantage of Descriptive Analysis is that data is easily statistically analyzed and graphically represented. Data analysis is performed averaging intensity data across panelists and replicates, once panel performance has been checked [21]. Thus, average intensity of all the evaluated sensory attributes provides a

sensory characterization of the products and enables the identification of relative differences among samples using statistical techniques such as analysis of variance [1]. It is important to highlight that average intensity of the evaluated samples are not absolute since they are related to the references used during panel training.

In order to get a graphic representation of the sensory profile of the samples, average information is usually represented using spider graphs, or in 2-dimensional plots from Principal Component Analysis (PCA). A spider graph (Figure 10.1) consists of a series of radial axes that represent the sensory attributes evaluated by the trained panel, and the average intensity of each sample for a given attribute is represented by the distance from the center of the graph. Meanwhile, PCA plots (Figure 10.2) represent the similarities and differences between the samples and their relationship with the evaluated sensory attributes.

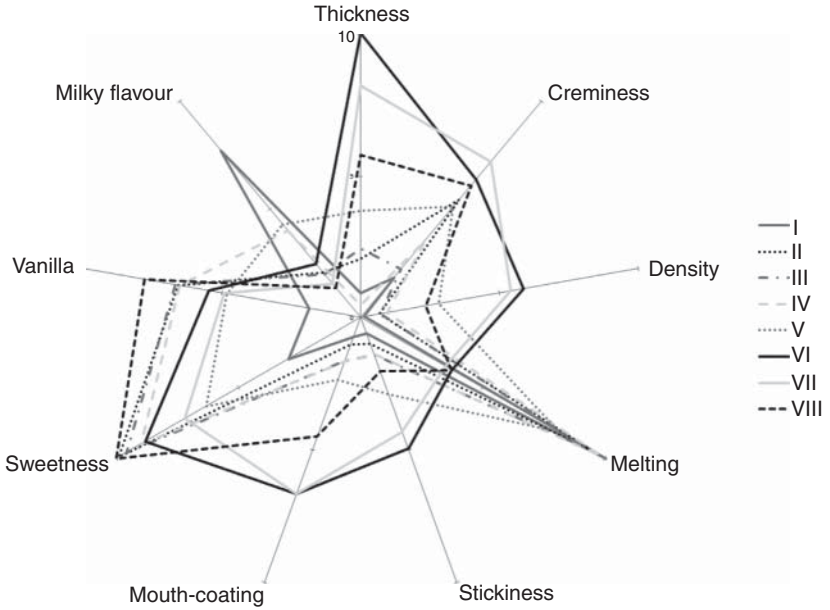

Figure 10.1 Spider graph for the evaluation of nine sensory attributes of milk desserts by a trained panel.

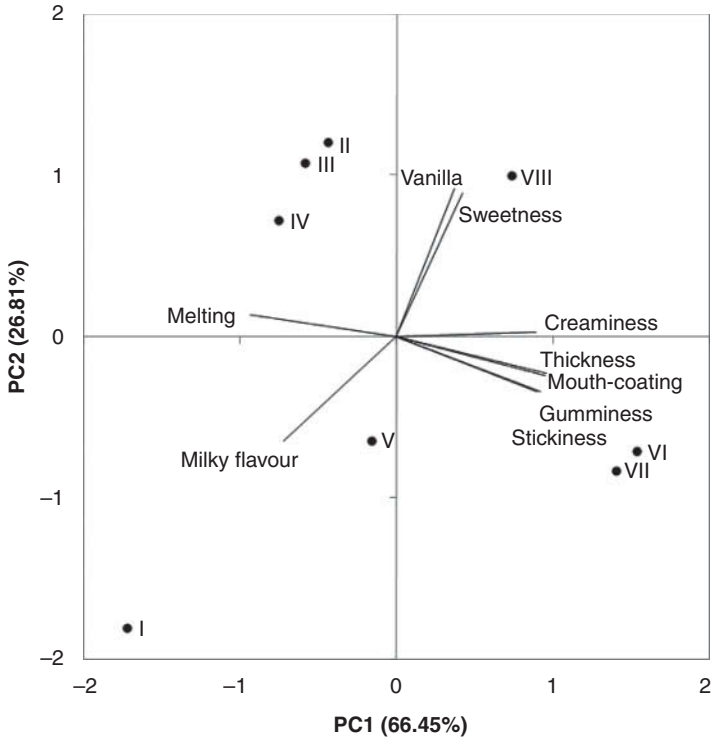

Figure 10.2 Bi-plot representation of the Principal Component Analysis (PCA) performed on average data from the evaluation of nine sensory attributes of milk desserts by a trained panel.

10.2 New Trends in Sensory Characterization of Food Products

10.2.1 Overview

Descriptive Analysis provides detailed, accurate, reliable and consistent results [2, 10]. In almost every major organization engaged in food research in Europe and the United States, as well as in many food companies, descriptive panels are used [22]. Due to the quality of the information provided, this methodology is expensive and time-consuming, which makes it difficult to apply in many everyday situations in the food industry where there are constraints in terms of time and resources [15].

In the first place, the vocabulary and associated panel training must be adapted to each specific type of product, which makes the time necessary to get reliable results from Descriptive Analysis relatively high. Considering that the times available for product development become shorter, on many occasions it is not possible to use Descriptive Analysis during the development of a novel product, which hinders the design of products with optimum sensory characteristics. Moreover, the fact that assessors have to complete an exhaustive training process and that the evaluations for a specific food category require several sessions, make it necessary for many food companies to maintain separate panels, since a single panel is not able to handle the evaluations of all the product categories produced [1]. These disadvantages limit the application of Descriptive Analysis in many specific applications; particularly in the case of small food companies and during the development of new food categories within a specific company [1]. Therefore, there is industrial pressure to develop alternative methods that obviate the need to train a sensory panel, or that at least reduce the training process.

Moreover, there is an increasing interest in gathering sensory information directly from the target consumers of food products instead of the more technical descriptions provided by trained assessors [23]. The most common approach to product optimization is to ask consumers to rate their liking of a large set of products and characterize the sensory properties of those products using a trained assessors' panel. Then, both data sets are combined using regression analysis to identify the sensory characteristics of consumers' ideal product [24]. In these approaches consumers are only asked about their liking, and therefore information about how they perceive the sensory characteristics of the products is not gathered. However, trained assessors could describe the product differently or take into account attributes that may be irrelevant for consumers [25]. Considering that the best way to understand consumer preferences might be consumer data [26], getting consumer feedback about the sensory characteristics of food products has become of great interest in the last decade.

In this context, several cost-effective methods for sensory characterization, alternative to Descriptive Analysis, have been recently developed. These methods do not require training, can be performed by trained assessors or consumers, and have been reported to be a good option when quick information about the sensory characteristics of a set of products is needed. There are

basically four types of methodologies: (i) methodologies based on the evaluation of specific attributes, (ii) methodologies that provide a verbal description of the products, (iii) holistic methodologies based on global similarities and differences among products, and (iv) methodologies based on the comparison of products with references.

10.2.2 Methodologies Based on Specific Attributes

This first type of methodology relies on the quantification of specific sensory attributes, as in conventional Descriptive Analysis. Its basic feature is that it saves time and resources by reducing to different extents the steps related to descriptor generation and panel training. Within this first type of alternative techniques for sensory characterization there are three main methodologies: intensity scales used by consumers, free-choice profiling, and flash profiling.

10.2.2.1 Evaluation of Sensory Attributes by Consumers Using Intensity Scales

A first alternative to reduce the time needed for training assessors for sensory characterization is asking consumers to rate the intensity of a fixed set of sensory attributes using scales, as it is commonly done with trained assessors in Descriptive Analysis. The main difference with the traditional approach is that descriptors are provided by the researcher and not generated by consumers, and that no training in attribute recognition or quantification is performed.

Despite the fact that this approach has not been traditionally recommended [1, 10], it has been recently considered as an alternative to the classical sensory profile provided by trained assessors [27, 28].

Many authors have reported that trained panels perform better than consumers or untrained panelists in terms of discriminative capacity and reproducibility, and that therefore training cannot be eliminated when evaluating the intensity of sensory attributes using intensity scales [15, 22, 29–31]. On the other hand, Moskowitz [32] stated that consumers are capable of validly rating the sensory aspects of products, based on the comparison with results from trained assessors. This author compared consumer ratings for 37 commercial sauce products with expert ratings and physical measurements, and concluded, based on the high correlation between

datasets, that consumers are able to assess the characteristics of food products. Hough [33] stated that the similarities in the performance of consumer and expert panels reported by Moskowitz [32] were due to the fact that the experts were not well trained and that only correlations between mean ratings were considered in the comparison.

More recently, studies have been published reporting that consumers are able to perform Descriptive Analysis, providing similar results as trained assessor panels. Husson *et al.* [27] analyzed results from two consumer panels from different geographies (218 and 124 assessors) for sensory characterization of 28 grape/raspberry beverages, by means of 10 attributes. Using analysis of variance and multiple factor analysis on mean ratings for the evaluated attributes, the authors concluded that both panels provided valid and comparable results.

Meanwhile, Worch *et al.* [28] studied the sensory profile of 12 commercial perfumes provided by a panel of 12 experts and by 104 consumers. The expert panel evaluated 12 attributes using 10 cm unstructured scales, whereas consumers evaluated 21 attributes using the same type of scale. In order to evaluate the reproducibility of the consumer panel two products were evaluated in duplicate. The authors used analysis of variance, correlation coefficients and multiple factor analysis to compare results provided by experts and consumers. They concluded that the two panels provided similar results in terms of discrimination, consensus, and reproducibility; reporting that the product spaces obtained from both panels were similar. However, it is important to take into account that in this study the expert panel was composed of students and teachers from an esthetic and cosmetic school, who were not subjected to any extensive training apart from one training session for the most difficult attributes.

Ares *et al.* [34] evaluated global and individual performance of a consumer panel for texture evaluation of milk desserts, and compared it with that of a trained assessor panel. These authors concluded that consumer and trained assessor panels showed similar discriminative capacity and reproducibility, being able to detect the same differences in the texture of the evaluated milk desserts. However, panel agreement and individual performance of the consumers were much worse than that of the trained assessors. In particular, consumer intensity scores were widely distributed along the scale and the majority of consumers were not able to

give scores that significantly discriminated among samples. Thus, it seemed that the lack of consensus in the consumer panel and the high variability in their evaluations were compensated by the large sample size and the fact that a small group of consumers had an outstanding performance in evaluating the characteristics of the products, even without training. For these reasons, using consumers for Descriptive Analysis using intensity scales would not be recommended, except for a couple of specific situations where the cost and time involved in the selection and training of the assessors might be higher than those needed to perform a consumer study with 50–150 participants. In particular, the evaluation of sensory attributes using intensity scales by consumers might be a good option in specific applications when food companies do not have a trained panel or when the product is not evaluated on a regular basis.

10.2.2.2 Free-Choice Profiling

Free-choice profiling is a method for sensory characterization of food products that was developed in the 80s to overcome some of the difficulties of conventional Descriptive Analysis [35]. The main assumption of this methodology is that assessors differ in the way in which they describe the sensory characteristics of food products [36]. Thus, instead of creating a consensus vocabulary and extensively training the assessors in the evaluation of specific sensory attributes, each assessor develops his/her own set of attributes and uses it to individually score the samples [37]. The main advantage of this approach is that assessors need little training since they just need to be capable of describing the sensory characteristics of food products and using line scales to quantify them according to their personal criteria [38]. For this reason, free-choice profiling is quicker and less expensive than Descriptive Analysis, and can be applied with both trained assessors and consumers [1, 39].

In order to overcome the difficulties faced by many untrained assessors to generate sensory terms to characterize the products [40], descriptor generation is usually performed using the repertory grid method [41]. Repertory grid consists of a simple personal interviewing technique that allows understanding which product characteristics are relevant for consumers and provides a list of sensory terms to be used for characterizing the products [42]. Products are arranged into triads (groups of three), and are presented to the

assessors in such way that two of the objects within the triad are arbitrarily grouped and separated from the third [42]. Assessors are asked to describe how the two grouped objects (A and B) are similar and different from the third (C) [41]. Once the assessor has elicited all the terms responsible for the similarities and differences between the groups, the researcher presents the remaining combinations within the triad (A and C vs B, B and C vs A) and once again asks the assessor to describe the similarities and differences. After the procedure is repeated for all the possible triads of products to be evaluated, all the sensory terms elicited by the assessor are placed together in a list next to unstructured scales. Then, assessors are asked to evaluate the products by rating the intensity of their own set of sensory attributes. It is important to highlight that in free-choice profiling each assessor evaluates his/her own set of sensory attributes, which are considered the most relevant for describing the products.

Due to the fact that assessors use an individual set of sensory attributes for their evaluations, data analysis should be carried out using Generalized Procrustes Analysis (GPA) [43, 44] followed by a principal component analysis [45], in order to get a consensus configuration from a set of individual data sets. GPA scales, translates, and rotates the data matrices of each panelist [43]. Each assessor's data is transformed into an individual spatial configuration, which is then matched into a consensus configuration, which provides information about the sensory characteristics that assessors used to distinguish the products, as well as a two- or three-dimensional representation of the similarities and differences between the samples [38]. Differences among products are explained considering the individual terms used by the assessors to describe each of the products.

Free-choice profiling (FCP) has been applied to a wide range of food products, including scotch whisky [46], beer [29], dark rum [47], vanilla samples [48], dry [49, 50] and cooked ham [51], carbonated soft-drinks [52], dairy products [53], coffee [54], and orange juice [55].

FCP is a simple and quick methodology that can provide relevant information to marketing and product development teams about the sensory characteristics of food products [56, 57]. However, results are generally not much actionable for product developers due to the fact that results mainly show the most important similarities and differences between the products, not providing information about the average intensity of the products or identifying subtle

differences between them [1]. Moreover, another disadvantage of the methodology is that many times the sensory terms used by some assessors to characterize the products are difficult to interpret [1] due to the fact that they are too personal. According to Deliza *et al.* [58] the terms used by consumers during the evaluation are closely related to their own individual experience and familiarity with the product.

10.2.2.3 Flash Profile

Flash profile is a sensory characterization technique that consists of a combination of free-choice profiling with a simultaneous comparative evaluation of the whole product set [59]. Flash profile was developed as a flexible and quick method that aims at providing information about the relative sensory positioning of a set of products [60].

The methodology is based on the assumption that comparing products is easier and more natural than evaluating them using intensity scales [61]. According to Dairou and Sieffermann [62] flash profiling should be performed with trained assessors or sensory evaluation experts in order to be able to better describe the products using discriminating and non-hedonic attributes.

Flash profiling is structured in two main sessions. In the first session assessors are presented with the whole set of products and are asked to generate their individual set of sensory terms which differentiate the products, avoiding hedonic terms [1]. Then, the researcher makes a list of the terms generated by all the assessors and shows it to each of them. The assessors are allowed to update their list by adding terms that they consider relevant but were not elicited by themselves or by replacing terms that are better adapted to the products. In the second session the assessors are presented the whole product set and are asked to rank the products according to their intensity of each of the attributes in their individual lists. At least three replications of the ranking session are recommended [62].

Data from flash profiling are commonly analyzed using Generalized Procrustes Analysis on ranking data, similarly to free choice profiling [1]. Using this analysis a consensus configuration is obtained, which allows the identification of similarities and differences among products, as well as their main sensory characteristics.

The main advantage of flash profiling is that information about the sensory characteristics of a set of products is gathered in a short time due to the fact that the phases of familiarization with the product space, attribute generation and evaluation are merged into a single step [61]. Considering that each assessor uses his/her own vocabulary to generate the list of sensory terms, the methodology allows a diversity of points of views [62]. Moreover, the fact that assessors have simultaneous access to the whole product set forces them to focus on the differences they perceive in order to generate only attributes which allow discriminating among samples [63]. For this reason, when the tested products belong to the same product category or to similar product categories, flash profiling has been reported to be more discriminating than conventional profiling [61].

However, flash profiling also has several disadvantages; the first of which is that this methodology is recommended for assessors with previous experience in sensory evaluation. As in free choice profiling the interpretation of the sensory terms is not always easy since assessors generate a large number of descriptors that lack definition and evaluation procedure [64]. Also, considering that all products should be evaluated simultaneously, in order to avoid fatigue, the number of samples to be evaluated in a single session is limited [1]. Besides, it might be difficult to apply flash profiling for shelf life testing or for evaluating products that require careful temperature control or that have intense and persistent sensory characteristics.

Flash profile has been applied to describe the sensory characteristics of different foods, such as red fruit jams [62], dairy products [61], pork sausages [65], commercial apple and pear purees [66], jellies [67], bread [68], wines [69], apple juice [70], hot beverages [60], fish nuggets [64], and ice tea [63].

10.2.3 Methodologies that Provide a Verbal Description of the Products

The second type of methodology is less analytic and rational. It aims at providing a verbal description of the products, which could be done by selecting words or phrases from a list (as in check-all-that-apply questions) or by providing a description of the products in an open-ended-question.

10.2.3.1 Check-All-That-Apply Questions

Check-all-that-apply (CATA) questions are a type of multiple choice question which have been extensively used in marketing research [71]. They have been recently reported to be a simple and reliable method to gather information about consumers' perception of the sensory characteristics of food products [72–74]. CATA questions consist of a list of words or phrases from which respondents should select all they consider appropriate to describe a product.

Products are presented to consumers in monadic balanced order and they are asked to check all the terms from the CATA question that they consider appropriate to describe each of the samples. There are no constraints on the number of attributes that could be selected by the consumers. The list of attributes included in the CATA question can include sensory characteristics (Figure 10.3a) but also terms related to non-sensory characteristics, such as usage occasions, product positioning and emotions (Figure 10.3b). The selection of the list of words or phrases included in the CATA question is one of the main challenges of the methodology. Sensory attributes can be selected based on the descriptors used by trained assessor panels to characterize the products or by using results from previous focus groups or consumer studies.

Data analysis from CATA questions is performed using Cochran's Q test [75] and Correspondence Analysis [25]. Cochran's *Q* test is used to evaluate if the consumers detected differences among

Figure 10.3 Examples of check-all-that-apply (CATA) questions composed of sensory (a) and non-sensory terms (b).

samples for each of the terms from the CATA question. Cochran's *Q* test is a nonparametric statistical test, which is used in the analysis of two-way randomized block designs to check whether k treatments have identical effects, when the response variable is binary. For each term of the CATA question a data matrix is created containing samples in the columns, consumers in rows and where each cell indicates if the term was mentioned or not (1/0 respectively) (Table 10.1).

Moreover, in order to get a representation of the samples, the frequency of mention of each term from the check-all-that-apply question is determined by counting the number of consumers who used that term to describe each sample (Table 10.2). Correspondence Analysis (MCA) or Multiple Factor Analysis is then performed on the frequency table containing responses to the CATA question [73,

Table 10.1 Example of the data matrix used for analyzing data from a term of a check-all-that-apply (CATA) questions using Cochran's Q test.

Consumer	Sample 1	Sample 2	....	Sample x
1	1	0	...	0
2	1	0	...	0
...	...	...	...	...
n	0	1	...	1

Each cell indicates if the term was mentioned or not (1/0 respectively) by each consumer.

Table 10.2 Example of the frequency table used for analyzing data from a term of a check-all-that-apply (CATA) questions using Correspondence Analysis.

Sample	Sweet	Creamy	....	Rough
1	34	45	...	0
2	22	0	...	14
...	...	...	...	...
x	17	43	...	3

Each cell indicates the number of times that a term was mentioned for describing each sample.

76]. This analysis provides a sensory map of the samples, which enables the determination of similarities and differences between the samples, as well as the sensory attributes that characterize them (Figure 10.4).

Despite the recent application of CATA questions to sensory characterization, it has been used for the sensory characterization of several food products such as snacks [72], strawberry cultivars [77], ice cream [74], milk desserts [73, 78], orange-flavored powdered drinks [76], and citrus-flavored sodas [79].

CATA questions have been reported to be a quick, simple and easy method to gather information about the sensory characteristics of food products. Ticking a box to select the sensory characteristics that describe a food product does not require much effort for consumers. Thus, CATA questions seem easier and have a smaller influence on liking scores than just-about-right or intensity questions [72]. Some

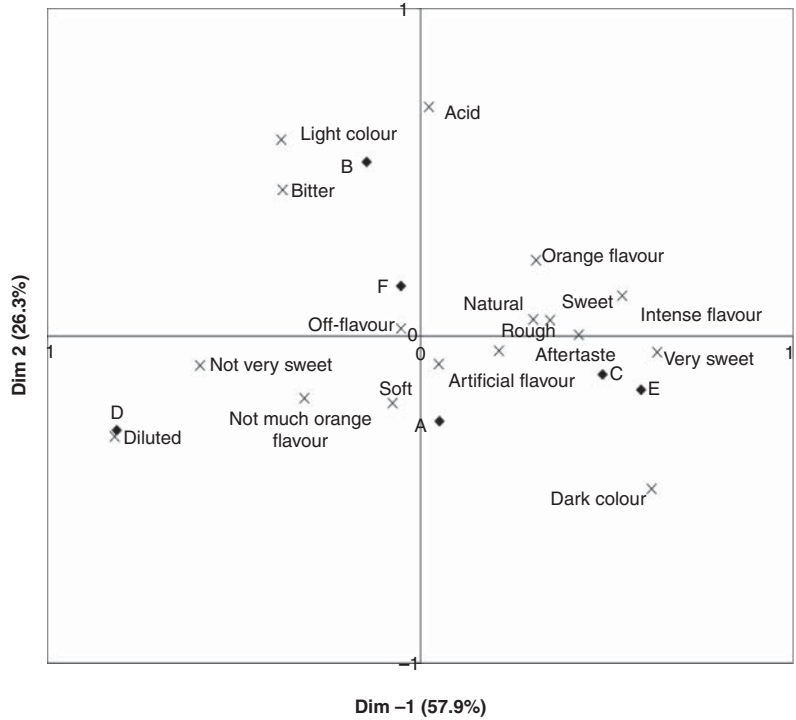

Figure 10.4 Representation of the first and second dimensions of a Correspondence Analysis performed on data from check-all-that-apply (CATA) questions for the sensory characterization of orange-flavored powdered drinks.

publications have suggested that the sensory maps generated by CATA questions are very similar to those from Descriptive Analysis with a trained assessor panel [74, 78, 80]. However, it is important to take into account that despite the fact that frequency of mention of the terms from CATA questions have been reported to be closely related to attribute intensity, they do not provide quantitative information since consumers only evaluate if a term is appropriate to describe a product or not. For this reason, data from CATA questions is qualitative and therefore might have smaller discriminative capacity than ranking tasks or intensity scales [74].

Another limitation of CATA questions is that it requires a relatively large number of consumers. Furthermore, it is worth mentioning that further research is needed to evaluate the influence of the number and type of terms in the sensory characterization provided by this methodology.

10.2.3.2 Open-Ended Questions

The application of open-ended questions to gather information about consumer perception of the sensory characteristics of food products was proposed by ten Kleij and Musters [25]. These authors allowed consumers to voluntarily write down remarks after their overall liking evaluations. Alternatively, Ares *et al*. [81] asked consumers to compulsorily provide up to four words to describe the samples, whereas Symoneaux *et al.* [82] gave consumers the option to freely state what they liked and/or disliked about the evaluated product. All these options enabled consumers to provide a description about the sensory characteristics of food products, which aims at understanding their perception and particularly what motivates their liking scores.

Consumer responses to open-ended questions are not subjected to any restrictions and therefore contain rich information that can underscore and complement quantitative findings from trained assessor panels [25]. However, the analysis of textual data is difficult, labor-intensive and time-consuming due to the inherent complexity of this type of data.

Consumers complete the open-ended question in their own style, without any specific guidance, even with typing, orthographic and grammatical mistakes, which makes it necessary to transform the data into precise sensory terms [82]. According to

Rostaing *et al.* [83] analysis of text data consists of the following stages: removing mistakes, elimination of connectors and auxiliary terms, identification of phrases and terms which make them up, lemmatization, regrouping synonyms, managing ambiguous words, and marking terms of interest for the researcher. The first step of the analysis usually consists of deleting stopwords, auxiliary terms and other irrelevant words. Then, words with similar meaning are grouped into the same category. This classification is usually performed independently by three researchers considering their personal interpretation of the meaning of the words and synonymy as determined by a dictionary. After individually evaluating the data, a meeting of the researchers is undertaken to check the agreement between their classifications. The final categories are consensually determined by the researchers. This triangulation technique has been used by other authors dealing with data from qualitative techniques [84]. Categories mentioned by more than 5% or 10% of the consumers are selected and frequency of mention of each category is determined by counting the number of participants that used each category to describe each product. After this, a frequency table or contingency table is constructed and analyzed. This data can be analyzed using Chi-square test and correspondence analysis. Ares *et al.* [81] carried out a global Chi-square test to study the independence between rows and columns, whereas Symoneaux *et al.* [82] used a Chi-square per cell test to identify significant differences among products and sensory characteristics within the contingency table. Data can finally be graphically represented using correspondence analysis [25, 81], which provides a 2-dimensional representation of the samples and the attributes.

This methodology has been used for sensory characterization in a limited number of food products: mayonnaise [25], milk desserts [81], and apples [82]. Results have shown that open-ended questions provide similar information to that obtained using Descriptive Analysis with trained assessor panels. However, it is important to highlight that the information provided by this methodology might not be precise enough compared to the traditional Descriptive Analysis performed with trained assessors, particularly when subtle differences between the products exist. It is important to highlight that, as in CATA questions, the data gathered by open-ended questions have little discriminative power due to their qualitative nature. Besides, words provided by consumers may be vague and

difficult to interpret, which makes data analysis tedious and difficult [81].

Open-ended questions can be considered an interesting complement to the traditional descriptive approach in order to gather the vocabulary used by consumers to describe the products, providing valuable information for marketing groups when developing communication strategies [81, 82].

10.2.4 Holistic Methodologies

Holistic methodologies are based on the assessors' perception of the global similarities and differences among products. They rely on the holistic or global perception of the products rather than on the analytical evaluation of specific sensory attributes. This consists of a first advantage of the methods since those aspects that are difficult to verbalize or define are not overlooked by assessors.

The most popular methodologies are free sorting and projective mapping, which are closely related to projective techniques. This type of technique is extensively used in psychology and is based on the assumption that the innermost feelings, beliefs, perceptions, attitudes and motivations of consumers can be uncovered by presenting them an unstructured and ambiguous stimulus [85]. In this context, projective techniques provide an indirect approach to consumers' perceptions, which allows researchers to transcend communication barriers and get information that is not affected by the instructions given to consumers before performing the evaluation [86].

10.2.4.1 Sorting

One of the most important operations in thinking is classification and categorization [87]. According to the *Merriam-Webster Dictionary* [88] a classification is a *systematic arrangement in groups or categories according to established criteria*. In the context of social sciences, sorting is a classification performed by a person [87]. Sorting has been extensively used in cognitive and social sciences as a systematic method for data collection, particularly when the objective is to uncover how people perceive a series of objects and what characteristics they attend to when making discriminations between objects [87, 89]. Sorting tasks have also been reported to be a powerful alternative to gather information about the sensory characteristics of food products in sensory and consumer science [90, 91].

The idea behind free sorting tasks is to measure the global degree of similarity among samples by grouping them according to their similarity.

Assessors (who can be trained assessors or consumers) receive the entire set of samples at once and are asked to try them and to sort the samples into groups according to their similarities and differences, using their own personal criteria. It is explained that two very similar samples should belong to the same group, whereas two samples that are clearly different should be placed in different groups. Assessors are usually told that they should sort the samples in at least two groups in order to avoid the trivial response of having all samples in the same group. In order to get information about the sensory characteristics responsible for the similarities and differences between the samples, a verbalization task is needed [92]. Thus, once the sorting has been completed, assessors are generally asked to provide descriptive words for each of the groups they formed [91, 93]. A typical classification provided by an assessor in a sorting task is presented in Figure 10.5.

Considering that assessors may find it difficult to provide a description of the sensory characteristics of each group of samples and that textual data is often difficult to analyze, Lelièvre *et al.* [94] provided the assessors with a list of predefined characteristics.

Different approaches have been proposed to analyze data from free sorting tasks. However, the idea behind all the approaches is to get a spatial map that represents the relationship of the samples in terms of their sensory characteristics [91]. The distance between

Group	Samples
1	735, 678, 098
2	057, 876
3	321

Description of the groups

Group N°1: *sour, bitter*

Group N°2: *creamy, soft, thick*

Group N°3: *off-flavour, disgusting*

Figure 10.5 Typical response of a single assessor to a free sorting task with six samples.

each pair of samples in the map is related to their degree of difference, i.e., if two samples are represented close to each other in the map they are very similar, and if they are represented far from each other they correspond to clearly dissimilar samples.

The most common statistical technique for analyzing sorting data is multidimensional scaling [91]. In this technique a similarity matrix is created by counting the number of times each pair of samples is sorted within the same group, as shown in Table 10.3. Then, either non-metric or metric multidimensional scaling (MDS) is performed on this similarity matrix in order to get a 2-dimensional representation of the samples, which provides a measure of the similarities between them. A typical sample representation from MDS is shown in Figure 10.6.

The main drawback of MDS is that information about the individual perception of the assessors is lost because individual data is transformed into a similarity matrix [91]. Thus, in this data analysis it is not possible to visualize if all the assessors sorted the samples similarly or if they had clearly different perceptions. In order to overcome this limitation, Abdi *et al.* [95] proposed the application of DISTATIS. This technique is a generalization of MDS, which allows analyzing 3-way distance tables. It takes into account individual sorting data. DISTATIS first analyzes the individual co-occurrences matrices of the participants, providing an optimal representation of the assessors which is based on their resemblance. Then, DISTATIS diagonalizes the linear combination of individual matrices to provide a consensus representation of the samples. Finally, the words used to describe product groups could be projected by using barycentric properties.

Table 10.3 Example of a similarity matrix containing data from a free sorting task.

	Sample 1	**Sample 2**	**....**	**Sample x**
Sample 1	50	27	...	0
Sample 2	27	50	...	13
...	...	...	...	...
Sample x	0	13	...	50

Each cell indicates the number of times that each pair of samples was placed together within the same group in the free sorting task.

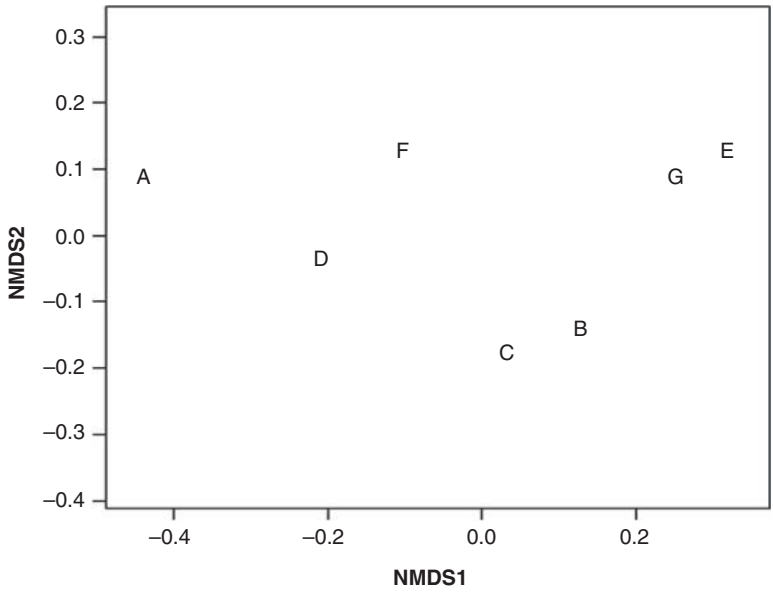

Figure 10.6 Typical sample representation of data from a free sorting task with seven samples using multidimensional scaling (MDS).

Moreover, Cadoret *et al.* [96] presented a different technique for analyzing sorting data, which is called FAST. This approach provides an optimal representation of the products based on Multiple Correspondence Analysis (MCA), and an optimal representation of the consumers based on Multiple Factor Analysis (MFA). In this technique all the consumers have the same importance when constructing the samples' map. An example of the sample representation from a free sorting task using FAST is shown in Figure 10.7.

The main advantage of DISTATIS and FAST is that they provide a representation of the consumers, which enables the visualization of individual differences and the identification of consumer segments with different perceptions. Moreover, by applying these techniques, the words used by consumers to describe the samples could easily be projected into the sample space. This last possibility improves interpretation, providing more actionable results.

Free sorting tasks have been extensively applied in sensory and consumer science to a wide range of products with different complexity such as cheese [91], drinking water [97], beers [98], red wine [99], yogurts [100], breakfast cereals [93], olive oil [101], and orange-flavored powdered drinks [76].

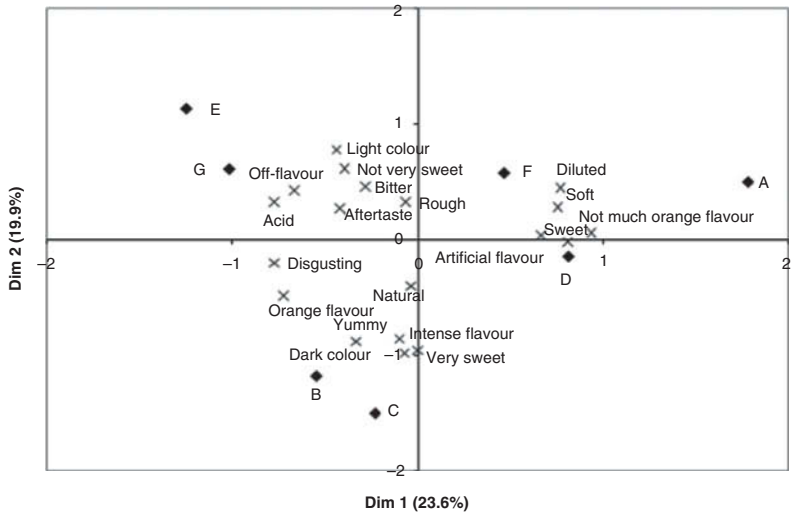

Figure 10.7 Representation of samples and descriptive terms from a free sorting task using FAST.

Free sorting has several advantages. Firstly, it does not require extensive training and produces little fatigue and boredom, which makes it appropriate for both trained assessors and consumers [102]. Besides, it does not require the use of scales or other quantitative systems [93]. Despite the fact that the method can be applied to a large sample set it is important to take into account that all samples should be presented simultaneously in a single session. Therefore, when working with complex fatiguing products, the number of products to be evaluated may be rather limited. Furthermore, one of the main limitations of free sorting tasks is that the descriptions provided by the assessors may be difficult to interpret in order to get actionable information.

10.2.4.2 Projective Mapping

Another alternative to traditional profiling is projective mapping, also known as Placing or Napping®. Risvik *et al.* [103] introduced the idea of projective mapping to quantify individual perception of overall similarity and dissimilarity among products. This methodology can be carried out with consumers or trained assessors, who are asked to provide a two-dimensional representation of a group of samples, according to their own criteria [26]. In this representation, the Euclidean distance between the samples is a measure

of their dissimilarity, in such a way that the smaller the distance separating two samples, the more similar they are. In this methodology assessors are asked to consider the product as a whole and to quantify the overall difference between pairs of samples [103]. As in any projective technique, the idea is to have a vague task which is not well defined, in order to get a simple and spontaneous response [26].

For performing a projective mapping task, all samples are presented simultaneously to the assessors, who are asked to place them on an A3 white sheet of paper (60 cm by 40 cm), according to the similarities or dissimilarities between them. Assessors are told to complete the task according to their own criteria since there are no right or wrong answers. It is also explained that two samples close together on the sheet correspond to very similar samples and that if they perceive two samples as very different they should locate them apart from each other. The positioning criteria and their importance are chosen on an individual basis by each assessor, which makes projective mapping a flexible and spontaneous procedure.

In order to understand the similarities and dissimilarities among samples in terms of their differences in their sensory characteristics, a description phase can be added to the projective mapping task [104]. This description phase is usually performed after the samples are placed on the white sheet.

For each assessor map, the X and Y coordinates of each sample are determined, considering the left bottom corner of the sheet as origin of the coordinate system, as shown in Figure 10.8. These data are analyzed using Generalized Procrustes Analysis (GPA) or Multiple Factor Analysis (MFA) [69, 104]. In MFA data analysis is performed on a data matrix composed of a set of columns that represent the X and Y coordinates of the samples in the sheets of each of the assessors for each of the evaluated samples (Table 10.4). In the MFA the coordinates of each assessor are considered as a group of two unstandardized variables, which enables the balancing of differences in how each assessor uses the horizontal and vertical coordinates [104]. The frequency table containing assessor descriptions is considered as a set of supplementary variables: correlation coefficients with the MFA factors were calculated and the variables are represented but they do not participate in the construction of these factors [104]. This analysis provides a consensus representation of the samples, a representation of the descriptions provided

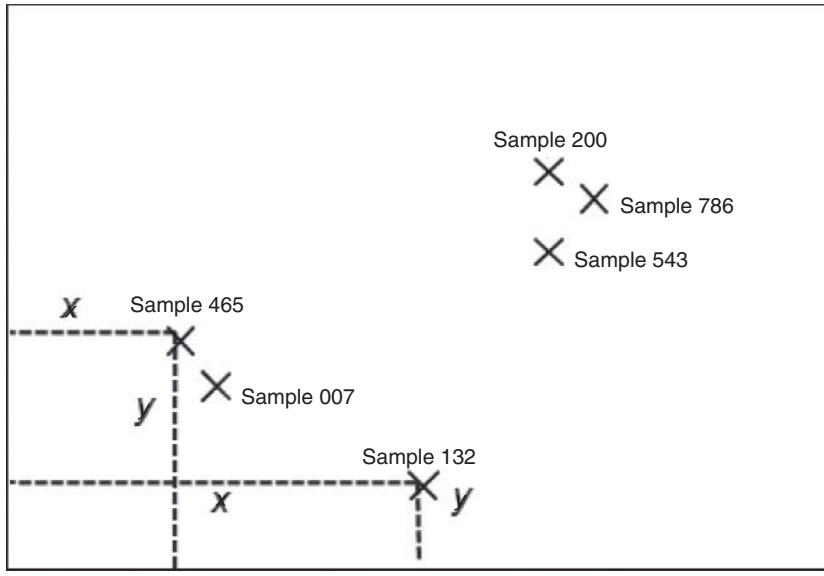

Figure 10.8 Example of sample representation of an individual consumer in a projective mapping task.

Table 10.4 Example of the data matrix used for analyzing data from projective mapping using Multiple Factor Analysis.

Sample	Assessor 1		Assessor 2			Assessor n	
	X1	Y1	X2	Y2	...	Xn	Yn
1	14.7	0.5	10.4	34.5	...	14.3	4.4
2	54.5	1.8	15.9	29.4	...	35.4	6.7
...	...	...	...	...	...		...
X	34.2	8.4	45.8	11.4	...	58.9	19.4

Each couple of columns Xi,Yi represent the coordinates of the samples in the map of consumer i.

by the assessors, and also a representation of the assessors, which indicates the similarity of their representations.

Projective mapping has been applied to a variety of food products, including chocolate [103], dried soup [26], snack bars [105], wines [69, 104], hot beverages [60], fish nuggets [64], milk desserts [73], orange-flavored powdered drinks [76], apples and cheese [106].

Projective mapping is a quick and simple technique which can be used with trained assessors, experts or consumers. As in free sorting tasks, projective mapping does not require the use of scales or other quantitative systems. On the other hand, one of the disadvantages of this methodology is that sometimes the differences between samples are difficult to explain due to the heterogeneity of assessors' descriptions. Moreover, in order to limit fatigue or adaptation, the number of samples presented should be limited to sets of approximately 10 samples which have to be simultaneously evaluated in a single session [107].

10.2.5 Methods Based on the Comparison with References

The fourth type of methodology for sensory characterization is based on the comparison of samples with products that are regarded as references and are readily available for evaluation. The main advantage of this approach is that it consists of a quick and easy methodology that enables the comparison of all products with fixed references, even if they are not evaluated in the same session. These methodologies are relatively novel and although they have been reported to provide interesting results, they have not been extensively applied yet.

10.2.5.1 Polarized Sensory Positioning

Teillet *et al.* [108] developed a quick method to explore consumers' perception of the sensory characteristics of water, which is called Polarized Sensory Positioning. This method is based on the comparison of food samples to a fixed set of reference products, or poles.

In this methodology, assessors (who could be trained or untrained) are asked to evaluate the degree of similarity of the samples with a set of standard products. In their initial proposal, Teillet *et al.* [108] selected three poles that represent three main tastes of mineral water and used unstructured scales ranging from "exactly the same taste" to "totally different taste" to measure how similar the taste of the sample was compared to the taste of each of the three reference products (poles), as shown in Figure 10.9.

Data analysis can be performed using two different approaches. In the first approach scores are considered as a measure of the distance from each pole. Then, data are averaged by sample and

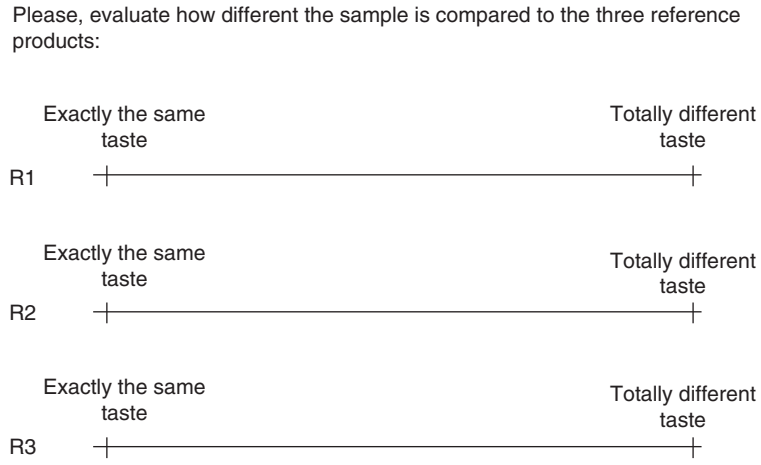

Figure 10.9 Example of an evaluation sheet used in Polarized Sensory Positioning to compare one sample with three reference products (R1, R2 and R3).

Table 10.5 Example of the data matrix used for analyzing data from projective Polarized Sensory Positioning.

Sample	Assessor 1			Assessor 2				Assessor n		
	R1	R2	R3	R1	R2	R3	...	R1	R2	R3
1	1.4	8.3	0.9	1.0	7.6	8.5	...	1.3	7.8	4.4
2	0.2	9.8	7.8	8.9	5.6	2.4	...	3.4	6.5	6.7
...	...	...	...	...	...	...	...	...	...	...
X	3.2	4.8	6.4	5.8	9.9	1.4	...	8.9	7.5	1.4

Each couple of columns R1, R2, R3 represent the degree of difference between a sample and each of the references (R1, R2 and R3, respectively) for each of the assessors.

analyzed using multidimensional scaling unfolding techniques [109] on the samples by poles matrix (Table 10.5), to get a two-dimensional representation of the similarities and differences among samples. In the second approach the poles are considered as descriptors and therefore data are analyzed by calculating average scores, whereas sample representation is obtained by principal component analysis [108].

The advantages of Polarized Sensory Positioning are the fact that it is an easy and quick methodology which could be performed with

trained and untrained assessors, and that it allows the comparison of data performed in different sessions.

Despite the fact that the only published application of this methodology to food products is related to mineral water, the methodology has a great potentiality due to the fact that it has been reported to provide similar results to Descriptive Analysis [108]. However, it is important to highlight that its application requires being able to have easily available and stable references. Besides, research is necessary to determine how reference samples should be selected, and particularly how many reference samples are necessary and what their characteristics should be. Besides, if no descriptive phase is performed after the task, information about the sensory characteristics responsible for the similarities and differences among products is not gathered.

10.2.5.2 Pivot Profile Method

The Pivot© Profile method was developed by Thuillier [110] to characterize the sensory properties of champagne. The method is based on the free description of the differences among samples and a reference product, which is called the "pivot."

Assessors are asked to provide a complete description of the attributes that they perceive as responsible for the sensory differences between the products and the pivot by completing two different columns, one described as "More" and the other as "Less." Thus, assessors should describe the sensory attributes that they perceive as less intense in the product compared to the pivot (e.g. less bitter), as well as those that they perceive as more intense (e.g. more acid). Assessors are asked to use only descriptive words and to avoid writing complete sentences. An example of the evaluation sheet is shown in Figure 10.10.

The first step of the data analysis is to analyze textual data to identify similar terms, which are grouped, as in open-ended questions. Then, the number of times that each term is positively and negatively mentioned is determined for each product and then the negative number is subtracted from the positive number. The final score is subjected to a transformation to get positive scores and the resulting matrix is submitted to Correspondence Analysis to get a sensory map of the evaluated products [110]. This analysis provides information on the main sensory characteristics of each product, as well as on the similarities and differences among the evaluated samples.

Sample:	Less	More
Appearance		
Odour		
Texture		
Flavour		

Figure 10.10 Example of an evaluation sheet used in Pivot Profile.

The Pivot Profile method is an easy and quick method, which can be applied with consumers or experts. This method is particularly recommended for wine experts since they are familiar with providing a verbal description of products. Besides, as in Polarized Sensory Positioning, it allows evaluating a set of products in separate sessions, which is particularly useful in complex products such as wine.

The main disadvantage of the methodology is that it requires a detailed analysis of textual data, which is difficult and time-consuming, as in the analysis of open-ended questions. Another disadvantage is that a stable reference product (pivot) is needed for the evaluation.

10.2.6 Comparison of the Methodologies

Several authors have compared conventional Descriptive Analysis and novel methodologies for sensory characterization of a wide range of food products with different complexity, ranging from mineral water [108] to wine [69] and fish nuggets [64]. In general, most studies have reported that, compared to Descriptive Analysis with trained assessors, novel methodologies provide similar information on the main sensory characteristics responsible for differences among products, as well as similar sensory maps [26, 36, 50, 60–62, 64, 74, 78, 80–82, 93, 98, 103, 108].

Despite the similarities in the information provided, it is important to highlight that the information provided by Descriptive Analysis is clearly different from that from novel methodologies for sensory characterization. Descriptive Analysis provides quantitative information about the average intensity of sensory attributes which enables the identification of significant differences among

samples in a specific attribute. However, it is not possible to gather this information using novel methodologies. From a statistical point of view, Descriptive Analysis is more robust than most novel methodologies, which makes it possible to identify subtle differences among products [64]. Besides, considering the assessors' extensive training, Descriptive Analysis is clearly more appropriate for comparing samples in different moments in time or different sample sets [60]. Besides, the interpretation of the sensory terms used in free-profiling, flash profile, open-ended questions, or holistic methodologies is in general a time-consuming, labor-intensive and difficult task due to the heterogeneity of consumers' descriptions, the large number of terms used and the lack of definitions and evaluation procedures. In particular, when differences among products lay in complex sensory attributes (such as creaminess or afterfeel) panel training for Descriptive Analysis is needed in order to get accurate information for product development [60]. For all these reasons results from Descriptive Analysis are much more actionable for product developers than those from novel methodologies; being that the latter is mainly useful when the objective is to identify the most salient attributes and the most important similarities and differences among products.

Despite the fact that Descriptive Analysis provides more accurate and reliable information in most cases, some clear advantages of novel methodologies can be highlighted. Firstly, the time associated with the implementation of novel methodologies for sensory characterization is much shorter than that needed to perform Descriptive Analysis, which makes them an interesting alternative in sensory and consumer science, particularly for those working in the industry. Another advantage of most novel methodologies is that they do not require using scales for evaluating the intensity of sensory attributes. Besides, they do not require consensus from the panel, which can potentially lead to some loss of information due to the fact that if the perception of the minority of the assessors differs from that of the majority, it is not taken into account [64]. The lack of need for consensus among panelists allows a diversity of points of views, which can lead to richer information [62]. Furthermore, in a small number of applications flash profile has been reported to provide more detailed information than Descriptive Analysis. Delarue and Sieffermann [61] stated that when working with similar products flash profile was more discriminating than Descriptive Analysis. Meanwhile, Albert *et al.* [64] reported that flash profile

with semi-trained assessors provided a more detailed description of the sensory characteristics of fish nuggets than Descriptive Analysis.

Novel methodologies differ in the way in which they gather information about the sensory characteristics of food products, which leads to differences in the information they provide [67]. Holistic methodologies are based on assessors' global perception of the products, which may enable the identification of the main attributes responsible for differences in their perception of the samples. In free sorting and projective mapping assessors focus their attention on the global perception of the products, which enables the identification of the most salient characteristics. On the other hand, on attribute-based methods assessors' perception is evaluated by attributes. This leads to differences in the information provided by similarity-based methodologies and those that rely on the evaluation of specific attributes.

Some authors have reported that similarity-based methods are less discriminative than those from methodologies based on the evaluation of specific sensory attributes, particularly when small differences among samples are considered. Veinand *et al.* [63] compared three methodologies (free-choice profiling, flash profile and projective mapping) for consumer profiling of lemon iced teas and reported that flash profile showed the highest discriminative ability, whereas projective mapping showed the lowest. Meanwhile, Albert *et al.* [64] concluded that flash profile provided more detailed information about the sensory characteristics of fish nuggets than projective mapping due to the fact that the latter was based on assessors' global perception of the products. When working with hot beverages, Moussaoui and Varela [60] reported that flash profile and free-choice profile provided richer vocabularies and more accurate sample maps than similarity-based methodologies such as projective mapping and free sorting. Moreover, these authors reported that untrained assessors were more repeatable when using flash profile than projective mapping or free sorting. Regarding the comparison of projective mapping and free sorting, Nestrud and Lawless [106] reported that despite the sensory maps provided by both methodologies were similar for apples and cheese, the identification of samples with similar sensory characteristics was easier to interpret for projective mapping than for sorting.

Apart from the reliability of the methodologies it is also important to take into account practical issues. Although most of the novel methodologies for sensory characterization have been reported

to provide similar results, they clearly differ in the difficulty that assessors encounter when completing the tasks. Holistic methodologies such as free sorting and projective mapping can be considered more intuitive and less rational than other methodologies based on the evaluation of specific sensory attributes. However, Ares *et al.* [76] reported that although consumers seemed to understand the projective mapping and sorting tasks, they found them much more difficult than CATA questions or intensity scales. Veinand *et al.* [63] reported that projective mapping was more difficult to perform with consumers than flash profile. According to these authors, when completing a projective mapping task consumers found it difficult to use the sheet of paper to locate the samples according to their similarities and differences. Also, Ares *et al.* [73] reported that projective mapping tasks required further explanation than CATA questions in order to assure that consumers understood the task.

Besides, regarding the time needed by assessors to complete the task, intensity scales, CATA questions, open-ended questions and pivot profile are less time-consuming than projective mapping, free-choice profiling, flash profile, and polarized sensory positioning. Whereas free-choice profiling and flash profile imply two separate sessions, one for generating the descriptors and a second one for evaluating the set of products, the rest of the methodologies can be performed in only one session. Besides, projective mapping and sorting tasks are more time-consuming than CATA or open-ended questions. According to Ares *et al.* [73], consumers needed between 5 and 15 min to complete a CATA question for characterizing the sensory properties of eight milk desserts, while it took consumers between 18 and 25 min to complete a projective mapping task.

According to these results holistic methodologies such as projective mapping and free sorting are more difficult and time-consuming for consumers. Considering that trained assessors with previous experience with sensory evaluation techniques can more easily understand these methodologies than naïve assessors, Veinand *et al.* [63] recommended the use of projective mapping only with expert panelists.

Another disadvantage of projective mapping is when paper sheets are used during the evaluations; it is tedious and tiresome for the researchers to measure the products' coordinates in the sheet of each assessor, particularly when a large number of consumers is considered [63].

Another clear difference between the methods is related to the number of samples that can be considered as a product set in an

experiment. Free-choice profiling, flash profile, free sorting and projective mapping request that all products should be evaluated by the assessors simultaneously due to the fact that comparisons between them are made. Therefore, in order to avoid fatigue and adaptation, the number of samples to be evaluated in a single session is limited, compared to other methodologies such as intensity scales, CATA questions or polarized sensory positioning. For this reason, it can be difficult to apply them when dealing with shelf life testing or the evaluation of products that require careful temperature control or that have intense and persistent sensory characteristics. In particular, polarized sensory positioning is appropriate to compare products over time with fixed reference products or when dealing with evaluations performed on different sessions. However, the criteria for selection of stable and easily available reference products should be carefully taken into account.

10.3 Conclusions and Recommendations

All the methodologies described in the present chapter consist of valid, reliable and quick alternatives for gathering information about the sensory characteristics of food products. They all have been reported to provide similar information to Descriptive Analysis performed with a trained assessor panel. However, it is important to highlight that the information provided by Descriptive Analysis is always more accurate due to the fact that assessors are extensively trained in the identification and quantification of sensory attributes. For this reason Descriptive Analysis seems more appropriate when the objective of the sensory characterization is to identify small differences between products or in the intensity of specific sensory attributes, as it happens in many cases during the optimization step of new product development.

However, when quick non-detailed information about the sensory characteristics of food products is needed, novel methodologies seem a very good alternative. They can be a valuable alternative to gather information about the sensory characteristics of food products for food companies that do not have the time or the resources to train a panel (which is common in developing countries such as Uruguay) for evaluating a specific product. In these cases the cost and time involved in the selection and training of the assessors may be higher than those needed to perform

a consumer study with 50–150 participants. Novel methodologies can also be interesting when conducting preliminary studies on the sensory characteristics of food products or when performing a screening for the selection of products or conditions for the design of a larger experiment.

Moreover, sensory characterization with consumers can only be considered complementary to Descriptive Analysis with trained assessor panels. This information can be extremely useful for uncovering consumer perception of food products, which can be valuable during new food product development or when designing marketing or communication campaigns. In this case an advantage of holistic methodologies, free-choice profiling and flash profile is that they enable the identification of consumers' vocabulary to describe the sensory characteristics of the products, while CATA questions and intensity scales should rely on previous studies to identify consumers' relevant terms.

The selection of a novel methodology for a particular application strongly depends on the type of assessors to be considered, practical issues and the specific objectives of the studies. However, when working with consumers the recommended approach would generally be to apply simple methodologies such as intensity scales, CATA questions, open-ended questions or pivot profile. Although verbal methodologies (such as CATA or open-ended questions, and intensity scales) have been reported to provide similar information than intensity scales, the former methodologies would be preferred due to their simplicity and ease of use, and the fact that they are more natural for consumers. On the other hand, when there is a trained panel available and quick information about the sensory characteristics of food products is needed, the recommended approach would be to apply flash profile, sorting, projective mapping or polarized sensory positioning due to their higher complexity. Holistic methods based on global similarity, such as sorting and projective mapping seem more appropriate when summarized sensory information is needed. Polarized sensory positioning or pivot profile seem a good option when there is strong interest in the comparison of new products with known ones, which can be considered reference products, or when the sensory characteristics of food products are to be compared over time.

Finally, it is important to take into account that most of the novel methodologies for sensory characterization have been used for a relatively short period of time and have been applied in a much

shorter number of applications than the traditional Descriptive Analysis. For this reason, further research on the applicability, reliability and reproducibility of new approaches for sensory characterization is strongly needed, particularly when working with complex products.

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11

Effect of Food Processing on Bioactive Compounds

Sarana Sommano

Science and Technology Research Institute, Chiang Mai University, Thailand

Abstract

Since there is an increasing concern with health, diet and lifestyle, particularly in the developed countries, the need for 'functional food' manufacturing is growing. Production of plant-based food as functional food started in 1970 and has been growing continuously. Methods of cooking can affect the stability of bioactive compounds in products containing them. Heat processing, for example, leads to ascorbic acid degradation but increases the level of phenolic content in vegetables such as tomatoes. Moreover, overheated products may also lead to unattractive colour and off-flavour due to the oxidation reaction of lipid and bioactive compounds themselves. However, heat treatment can increase lycopene bioavailability via isomerisation. Drying and/or freezing of plant materials are vitally important to preserve aroma and flavour. These may be followed by cooking or heat processing such as pasteurisation. Pasteurisation prolongs shelf life of the products by the destruction of spoilage microorganisms and/or the inactivation of the enzyme. This chapter reviews the effect of food processing on the bioactive compounds during production of food-based bioactive compounds.

Keywords: Antioxidant, cooking, functional food, pasteurisation, phenolic compounds, reactive oxygen species, stability

**Corresponding author*: sarana.s@cmu.ac.th

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11.1 Bioactive Compounds

11.1.1 Reactive Oxygen Species (ROS)

Since the mid-1960s after the description of reactive oxygen species (ROS) was released, people have been aware of diseases such as cancers, cardiovascular disease and dementia that cause an increasing numbers of deaths each year. Many studies have confirmed the predominant biological toxicity of ROS links to those diseases [1–3].

ROS can be classified according to the way they are formed [4]; the superoxide anion radical (O_2^-), the peroxide anion (O_2^{2-}), and singlet oxygen ($^1O_2$) are created from molecular oxygen (O_2) by obtaining a single electron, or the realignment of the electron spins. The hydroxyl radical (•OH) is formed by dismutation of peroxide, which is catalyzed by Fe^+.

Antlovich *et al.* [5] described the mechanism of lipid oxidation, a good example of ROS introducing human diseases. The most common pathway for lipid oxidation is non-enzymatic free radical-mediated chain reaction which was described in three steps namely; initiation, propagation and termination. External agents such as heat, light, metal ions or ionizing radiation are involved in the initiation step.

$$\textit{Initiation}: LH + R^{\bullet} \longrightarrow L^{\bullet} + RH$$

Firstly, the radical ($L^{\bullet}$) is formed from the substrate molecule which is normally a lipid substrate. Then the radical rapidly reacts with oxygen to form a lipid peroxyl radical ($LOO^{\bullet}$). This peroxyl radical can continuously oxidize with other lipid substrates and eventually form lipid hydroperoxides (LOOH). This is the propagation stage.

$$\textit{Propagation}: L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}$$

$$LOO^{\bullet} + LH \longrightarrow LO^{\bullet} + LOOH$$

The next step is the breakdown of LOOH and the formation of a wide range of compounds viz., alcohols, aldehydes, alkyl formats, ketones and hydrocarbons and radicals including the alkoxyl radical ($LO^{\bullet}$).

Branching: $LOOH \longrightarrow LO^{\bullet} + HO^{\bullet}$

$$2LOOH \longrightarrow LOO^{\bullet} + LO^{\bullet} + H_20$$

The last step is the termination which is the combination of radical substance to non-radical substance.

Termination: $LO^{\bullet} + LO^{\bullet}$

$LO^{\bullet} + LOOH^{\bullet}$ → non radical product

$LOOH^{\bullet} + LOOH^{\bullet}$

11.1.2 Antioxidant Defenses Against ROS

The oxidation reaction *in vivo* induces the degenerative and age-related diseases, e.g., cancers. The importance of the antioxidants present in foods is apparent for both preserving food and supplying health benefits. Antioxidants have various lines of defense. The first is that antioxidants can retard the rate of oxidation by removing the substrate or quenching singlet oxygen thereby inhibiting the formation of ROS, or by sequestering metal ions and reducing hydroperoxides and hydrogen peroxide [5]. The second role is that the antioxidants work as free radical-scavengers. Vitamin E, for example is one of the major lipophilic free radical-scavenging antioxidants. This compound scavenges free radicals and inhibits chain initiation. The mechanism is shown as in the following chain reaction [6]. Primary antioxidants, AH, usually react with free radicals ($LOO^{\bullet}$, $LO^{\bullet}$) when present in trace amounts [5]. Table 11.1 illustrates the function of antioxidant against ROS.

$$L^{\bullet} + AH \longrightarrow LH + A^{\bullet}$$

$$LOO^{\bullet} + AH \longrightarrow LOOH + A^{\bullet}$$

$$LO^{\bullet} + AH \longrightarrow LOH + A^{\bullet}$$

Moreover in the propagation step, the antioxidant free radical may react with other free radicals and finally, form the peroxy antioxidant compounds.

Table 11.1 Antioxidant defence againsts ROS.

Carcinogens	**Antioxidants**
Pollutants, tobacco, food , UV, infection agents, etc. ↓	β-carotene, lycopene, vitamin C,α-tocopherol, polyphenolic compounds, etc.
Endogenous substances	Inhibition ← Scavenge →
Metal, photosensitizers, reducing agents ↓	
Reactive oxygen/nitrogen species (ROS)	
↓ DNA damage	↓ Inhibit the formation of ROS or work as radical scavengers

Source: adapted from [6, 7].

$$A^{\bullet} + LOO^{\bullet} \longrightarrow LOOA$$

$$A^{\bullet} + LO^{\bullet} \longrightarrow LOA$$

11.1.3 Bioactive Compounds or Natural Antioxidants

Researchers have investigated antioxidants and found that various kinds of antioxidants can protect humans from oxidative stress [2, 8, 7, 9]. They have suggested that though synthetic antioxidants have been developed and used in practice, natural antioxidants can be more potent, efficient and safe.

Fruits and vegetables are a good source of natural antioxidants. Basu *et al*. [4] mentioned that consuming these natural antioxidants potentially reduced risks in having cancer and cardiovascular disease. For example, vitamin E in fruits and nuts is the major lipid-soluble antioxidant present in low density lipoprotein (LDL) and can prevent the formation of lipid peroxides. Vitamin C in fruits and vegetables can also scavenge free radicals in cytoplasm [4, 10]. However, although β-carotene, a vitamin A precursor, is contained in LDL, the antioxidant mechanism has yet to be known [4].

The other bioactive compounds found in fruits and vegetables such as flavonoids are also beneficial. Aged garlic extract which

contains high flavonoids such as s-allylcysterine and N-alpha-(1-deoxy)-D-fructos-1-yl)-L-arginine was studied in men and was shown to improve endothelial function, decrease LDL oxidation, inflammatory factors, and slow the development of experimental atherosclerosis [4]. The well known natural antioxidants found in natural produce are described below.

11.1.3.1 Carotenoids

Carotenoids are plant pigments found associating with chlorophylls in plant cell plastids. They impart the red and yellow colors to fruits and vegetables. Carotenoids are the source of unsubstituted β-ionone ring which then can be oxidized to yield retinaldehyde and reduced to retinol or oxidized to retinoic acid, the active form of Vitamin A. The human body can absorb about 70% to 90% of dietary retinol even at high consumption levels of the carotenoid. However, not all carotenoids contain an unsubstituted β-ionone ring and therefore cannot act as precursors of retinol, e.g., lycopene [11]. However they may still exhibit antioxidant activity. Common carotenoids in plants are:

a. **Beta-carotene:** This compound imparts the yellow pigmentation to plants. It functions as a precursor to vitamin A which is a highly effective quencher of singlet oxygen and a free radical scavenger. Beta-carotene can be found in plants, for example, sweet potatoes, carrots, tomatoes, apricots, prunes and oranges [11]. The evidence has illustrated that taking vitamin A and β-carotene decreases the incidence of cancers of bladder, lung, upper gastrointestinal tract and breast [7].
b. **Lycopene:** Lycopene is one of the bioactive compounds classified in the carotenoid group. Synthesized by plants and microorganisms, the function of this compound is to protect cells against photosensitization and to serve as a light-absorbing pigment during photosynthesis [12]. Heber and Lu [13] indicated that lycopene can trap singlet oxygen and reduce oxidative stress, which also are linked to the reduction in risk for cancer. Lycopene, moreover, can be converted to β-carotene by enzyme lycopene cyclase [13].

The highest consumption of lycopene in the United States is derived from tomato products, as shown in Table 11.2. Fresh tomato contains less lycopene than tomato sauce or processed tomato [14]. Lycopene found in fresh fruits and vegetables is in the form of all-*trans* geometrical configuration. However, the active configuration is *cis*-isomer which can be found in plasma and tissue samples in significant amounts [15]. Interconversion of these two isomers is due to thermo-energy, absorption of light or by involvement in specific chemical reactions. Studies have shown that heating tomato and the bench-top preparation of tomato products increases the concentration of *cis*-isomers [12, 14]. However, the common heat treatments did not cause any change. Sommano *et al.* [14] and Nguyen and Schwartz [15] also suggested that heating tomato in the presence of lipid could improve the bioavailability of lycopene.

Table 11.2 Lycopene content in tomato and tomato products.

Food	**Type**	**(mg/100 g fresh wt.)**
Ketchup	Processed	16.60
Papaya	Red, fresh	2.00–5.30
Pizza sauce	Canned	12.71
Pizza sauce	From pizza	32.89
Rosehip puree	Canned	0.78
Salsa	Processed	9.28
Spaghetti sauce	Processed	17.50
Tomatoes	Red, fresh	3.1–7.74
Tomatoes	Whole, peeled, processed	11.21
Tomato juice	Processed	7.83
Tomato soup	Canned, condensed	3.99
Tomato paste	Canned	30.07

Source: Hadley *et al.* [16].

11.1.3.2 Vitamin C (Ascorbic Acid)

Not only does vitamin C prevent scurvy, it also generates ascorbate which assists the alteration of vitamin E to the active form. Ascorbic acid per se can quench free radicals and singlet oxygen [4].

Ascorbic acid can function by regenerating or restoring antioxidant activity or work as a synergist accompanied with the ability to act as an inhibitor during oxidation. In addition, it is an effective radical scavenger of superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen. The natural structure of vitamin C found in fruits and vegetables is L-ascorbic acid and dehydroascorbic acid. There is some evidence that vitamin C may provide a positive effect against illnesses such as cancer and play an important role in heart disease and other disabilities of aging [17].

Eitenmiller *et al.* (18) explained the factors which lead to a loss of ascorbic acid stability, that is, for example, the pH range. Maximal stability of vitamin C usually exists between pH 4 and 6. Oxygen availability, transition metal catalysis and the presence of ascorbic acid oxidase also affect the degradation rate. Losses while cooking depend upon degree of heating. Leaching or blanching and the exposure of surface area to water and oxygen, can increase the rate of loss. Ascorbic acid oxidase action can also be accelerated while blanching as the heat is slowly increased. Therefore, steaming vegetables is the recommended method for preserving ascorbic acid content [18].

11.1.3.3 Vitamin E (Tocopherol)

Vitamin E can act as an antioxidant as it retards or inhibits the oxidation of unsaturated fatty acid, and works as a free radical trapping antioxidant in membranes [11]. In comparison to other lipid-soluble antioxidants viz., γ-tocopherol, β-carotene, and α-carotene, LDL carries about 50% of the circulating vitamin E which is much greater than the other lipid-soluble antioxidants. Vitamin E can be found in vegetable oil, especially in wheat germ oil which has a high α and β tocopherol content.

Vitamin E is most stable in light-reduced conditions, in the absence of heat, at moderate pH, and is susceptible to lipoxidase reactions, various metals, primarily iron and copper, and the presence of free radicals which induce autoxidation in the absence of oxygen; tocopherol is stable to heat and alkali conditions [18].

11.1.3.4 Phenolic Compounds

Phenolic compounds are characteristically presented in higher plants. They act as potential antioxidants in relatively small amounts, whereas at high concentrations they can behave as oxidative substrates [5]. Due to their aromatic ring bearing one or more hydroxyl groups adjacent or ortho to each other, as well as a number of other substituents, phenolic compounds are good candidates for enzymatic browning [19, 20]. Plant polyphenols can be categorized into non-flavonoids and flavonoids depending on the complexity of the molecule [19].

11.1.3.4.1 Non-flavonoids

The non-flavonoids are simple-molecule polyphenols, a group that includes stillbenes and phenolic acids (Figure 11.1). The latter group

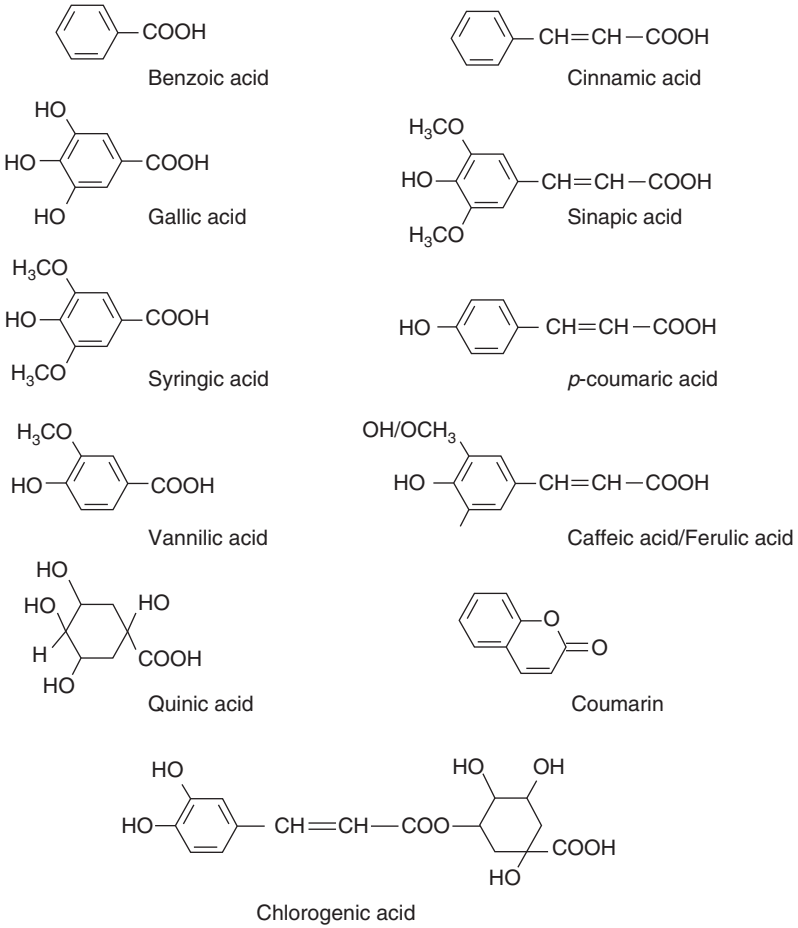

Figure 11.1 Common non-flavoniod compounds. Source: Marshall *et al.* [20].

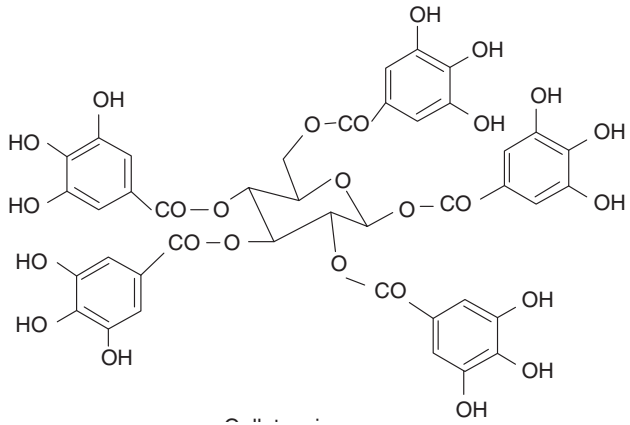

Figure 11.2 Complex structure of non-flavonoid compound. Source: Cheynier [19].

can be divided into benzoic acid (C1-C6 skeleton) and hydroxycinnamic acids (C3-C6 skeleton). The complex derived forms of stillbenes and phenolic acids are also included in the non-flavonoid group (Figure 11.2) (i.e., stilbene oligomers, gallotanins, ellagitanins and lignins) [19].

11.1.3.4.2 Flavonoids

Flavonoids belong to a group of polyphenols called diphenylpropanes. Their basic structure consists of two benzene rings joined together with an aliphatic chain, which is presented in a pyran form or, less commonly, a furan ring. Members of the flavonoid group are flavonols, flavones, flavonones, flavan-3-ols and anthocyanins (Figure 11.3).

Catechins (Flavan-3-ols)

Catachins are the most common polyphenols in green tea and are found in the forms (+)-catechin, (–)-epicatechin, (–)-epigallocatechin, (–)-epicatechin gallate, and (–)-epigallocatechin gallate (EGCG), as shown in Figure 11.4. Beside its involvement in H_2O_2 generation during the enzymatic reaction of POD, catechin is an oxidative substrate for plant polyphenol oxidase. The oxidative coupling profile of (+)-catechin by PPO extracted from grape, for instance, has been reported to yield noncolored product at pH below 4, while yellow compounds were seen at higher pH [22].

Anthocyanins

Anthocyanins are plant pigments representing blue, red and purple colors in fruits, vegetables and flowers. This group of pigments

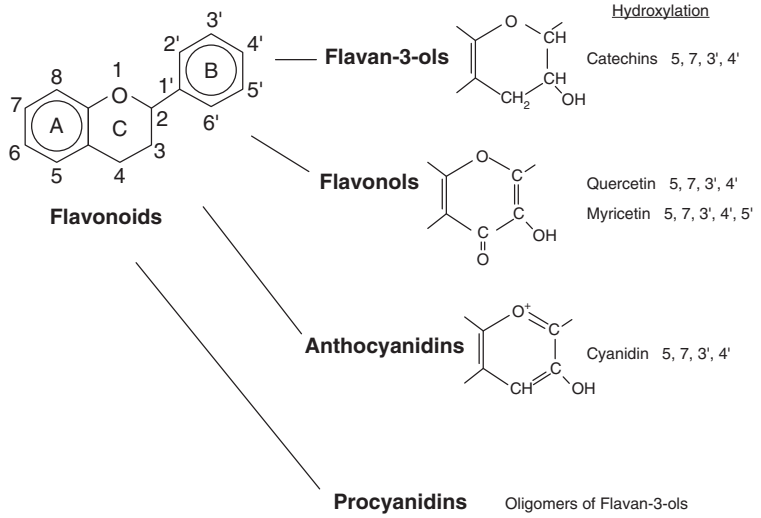

Figure 11.3 Structure of flavonoid and its members. Source: Frankle [21].

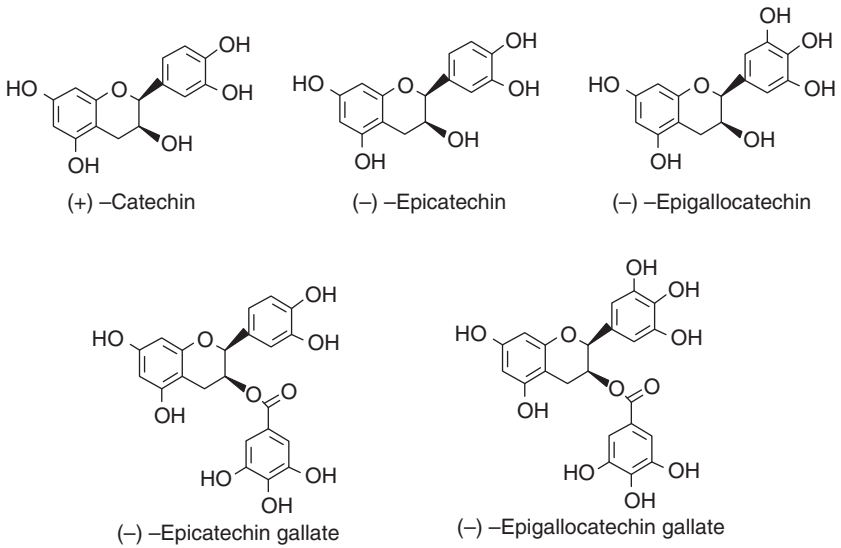

Figure 11.4 Structure of common catechins in green tea. Source: Uyama and Kobayashi [23].

can be found in almost every part of plants. Within the anthocyanin group, they differ from each other in the number of hydroxyl groups, the nature and number of sugars attached to the molecule, the position of this attachment, and in other substituents attached to sugar molecules. As shown in Figure 11.5, pelargonidin, cyanidin,

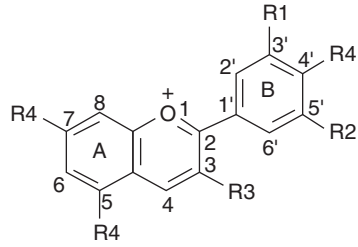

Figure 11.5 Structure of six common anthocyanins, where R3 and R4 = OH; pelargonidin;R1, R2=H; cyaniding, R1 = OH, R2 = H; peonidin, R1 = OCH_3, R2 = H; delphinidin; R1, R2 = OH; petunidin, R1 = OCH_3, R2 = OH and malvidin, R1, R2 = OCH_3. Source: Adapted from Kong *et al.* [24].

peonidin, delphinidin, petunidin and malvidin are the six most common anthocyanins in higher plants [24].

11.1.4 Other Significant Bioactive Compounds

Isoflavones

Isoflavones are groups of phenolic compounds found in legumes and soy. Isoflavones function as phytoestrogens which increase the level of oestrogen in some tissues and retard the effects of oestrogen in others. The pro-oestrogenic effects could help the body maintain bone density and improve blood lipid profiles (cholesterol levels) while anti-oestrogen effects protect the body from hormone-related cancers such as breast and prostate cancers [25].

Glucosinolates and Isothiocyanates

Dietary glucosinolates are converted to isothiocyanates by the enzymatic action of myrosinase [26]. This group of bioactive compounds is found in cruciferous vegetables, such as broccoli, cabbage and kale. They confer a number of antioxidant activities, for example, working as biotransformation enzymes in order to eliminate drugs, toxins and carcinogens. They also play an important role in preventing cancers [25, 26].

Lignans

Lignans are groups of phenolic compounds formed by the action of intestinal bacteria with lignan precursors. They can be found in seeds, whole grains, legumes, fruits and vegetables. As they are considered to be phytoestrogens, they can reduce risk of hormone-associated cancers (breast, uterine, ovarian and prostate) [25].

11.2 Processing of Foods Containing Bioactive Components

11.2.1 Effect of Postharvest Handling Methods and Shelf Life Determination

To retard postharvest degradation, maintaining food within its optimal conditions is important. Florkowski *et al.* [27] indicated that to keep fruits and vegetables in optimum condition, the environment needs to be maintained at optimal temperature and relative humidity (RH). For this, use of chemical preservatives or gamma irradiation treatment may also be required in some circumstances.

The effects of postharvest practices on the shelf life of raw materials, i.e., storage conditions after harvest, need to be evaluated. The mode of transportation is also very important. For example, shipping or road transport may take a long period of time and therefore considerably longer storage time may be required [28].

A number of research projects have been focusing on the effect of storage conditions on quality of food. Dhemre and Waskar [29] suggested that storage of mangoes in a cooling chamber could maintain the quality and market acceptability. Boukobza and Taylor [30] worked on the effect of pre- and postharvest treatments on the level of volatile compounds in fresh tomato quality and found that varietal and seasonal factors have a significant effect on the loss of volatile compounds; whereas the study of chilling storage caused a reduction in the levels of volatile components, as did short-term, high-temperature storage (45°C for 15 hours).

McGlynn *et al.* [31] studied sanitizing dip as postharvest treatment on the quality of fresh-cut watermelon. They found that a pre-cut sanitizing dip reduced about one to two log cycles in initial aerobic and coli form bacterial counts. This is expected to extend the shelf life of fresh cut melon when stored at 4°C for up to 14 days.

However, there is limited information on the effect of postharvest treatments on the bioactive components of fruits and vegetables, especially in native plants [32]. Sommano *et al.* [33] indicated to maintain a good level of bioactive compounds from native food ingredients throughout food processing, freeze drying is recommended.

11.2.2 Effect of Processing

While processing brings numerous benefits to food, for example, it makes food healthier, safer and tastier; the method of preservation may also have deleterious effects.

11.2.2.1 Effect of Heat Processing

Heating is another physical factor. Pasteurization can destroy vitamins, therefore the addition of vitamins post-processing may be required, for example, the loss of vitamin C and β-carotene in fruit juices. However, de-aeration during the process may minimize the loss [34]. The existence of transition elements such as Cu^{+2} and Fe^{+3} also lead to the oxidation of ascorbic acid to diketoglulonic acid which results in a loss of bio-potency.

Sterilized milk has appreciable changes in ascorbic acid and vitamin B_{12} contents following processing (Table 11.3) but the reduction is much less for UHT milk than for in-bottle sterilization. In fruits and vegetables, heat treatments also affect soluble vitamin levels, particularly ascorbic acid. However, in canned fruits and vegetables, even

Table 11.3 Changes in nutritive value of milk after UHT and in-bottle sterilization.

Nutrients	Loss (%) on processing	
	UHT	In-bottle
Thiamin	10	35
Ascorbic acid	25	90
Vitamin B12	10	90
Folic acid	10	50
Pantothenic acid	0	0
Biotin	0	0
β-carotene	0	0
pyridoxine	10	50
Vitamin D	0	0
Whey protein (denaturation)	12–40*	87
Lysine	-	10
Cystine	-	13
Biological value	-	6

* Direct UHT at 135°C for 2 seconds (12.3%) and indirect UHT at 135°C for 2 seconds (40.3%). Source: Fellows [34].

though there are some losses due to heat processing, some vitamins are transferred into the brine or syrup which is consumable [34].

Generally, most bioactive components are sensitive to light, heat, pH and oxygen. Franke *et al.* [35] reported that the processing of fruits and vegetables consumed in Hawaii by heating resulted in loss of vitamin C from 28% to 63%. Gahler *et al.* [36] and Sommano *et al.* [13] also found that the vitamin C content of processed tomato products decreased during heat processing. However, the total phenolic content and the antioxidant capacity increased.

Moreover, they also noted that the materials of the stewing pan have some effect on the loss of vitamin C. Double-based stainless steel pans retained more vitamin C than Teflon and pyrex pans. Rumm-Kreuter and Demmel [37] recommended that to preserve the vitamin content of processed vegetables, steaming is strongly suggested for products which require a short cooking period, spinach for example. On the other hand, for products which need a longer cooking time, bean soup for example, pressure cooking retained much higher ascorbic acid content than steaming.

In the processing of tomato pulp, the tomato pulp needs to be concentrated by heating at 100°C under atmospheric pressure. Sharma and LeMaguer [38, 39] found that when concentrated tomato pulp was heated, the rate of lycopene degradation was higher than it was when heating unconcentrated pulp. The *cis*-lycopene isomer has been detected in human plasma and tissues. Heating tomato juice can increase the content of this isomer and, therefore, lead to an improvement in overall lycopene bioavailability in humans. Nguyen *et al.* [15] indicated that using excessive amounts of heating induced the alteration of lycopene *trans*-isomers in fresh vegetables to lycopene *cis*-isomers. They also suggested that thermal processing of tomato products with the presence of oil may enhance the bioavailability of lycopene.

In a study of the retention of folate in various foods within the United Kingdom, McKillop *et al.* [40] found that steaming was preferred to boiling green vegetables, such as spinach and broccoli, and could be promoted as a means of doubling folate content. They also recommended that boiling the potato with the skin on could retain a greater amount of folate than cooking without the skin.

Gayathri *et al.* [41] studied the effect of the addition of acidulants on β-carotene levels in vegetables during cooking. They found that the presence of acidulants, tamarind and citric acid, which bring about a reduction in pH of up to one unit at the concentration at which they are included in the diet, improved the retention of β-carotene during heat processing.

11.2.2.2 Effect of Freezing

11.2.2.2.1 Freezing

Freezing damage is an irreversible change in the tissue that becomes apparent after thawing. Solute concentration damage refers to the reduction of water activity (Aw) in food, leading to an increase in pH. The increase in ionic concentration and strengths can damage the food and alter it permanently [34].

Nursal and Yucecan [42] showed that after thawing frozen peas but before boiling, the vitamin C content loss was about 40.8%. By comparison, boiling frozen peas without thawing led to a decrease of Vitamin C of about 25%, much less than if thawed before boiling.

Fellows [34] suggested that dehydration damage occurs from an increase in solute concentration in the unfrozen portion of the food. This leads to an osmotic transfer of water from the cell interior to the external environment so that the cell is dehydrated and the volume of the cell is changed. Mechanical damage can also occur to fragile cellular structures when the flexible cell components are stressed in areas where ice exists.

The change in bioactive components of frozen food normally happens when in storage, during which time the pigments of frozen fruits and vegetables are deteriorated. For example, chloroplasts and chromoplasts are broken down and chlorophyll is slowly degraded to brown pheophytin. The change in pH of frozen fruits due to precipitation of salts also alters the color of anthocyanins [34]. During the storage of frozen fruits and vegetables at -18°C, the water soluble vitamins are lost. Vitamin C is most affected with a loss of 10–50% in fruits and vegetables (Table 11.4). Other vitamin losses are due to drip loss.

11.2.2.2.2 Freeze drying

The main aim when preserving food containing bioactive compounds is to retain the nutritional quality of the product, as well as the sensory characteristics. Without heating food, a similar preservative effect can be achieved by reducing water activity so that the bioactive compounds are better maintained [34]. There is much evidence for the benefits of vitamin retention in freeze dried fruit and vegetable powders. Another advantage is that freeze dried products provide natural color and flavor characteristics so that these products can replace one or more other artificial ingredients along with supplying the fortification of natural vitamins and minerals [34].

Table 11.5 shows losses of vitamins during freeze drying. The table illustrates the substantial changes in ascorbic acid content of

Table 11.4 Vitamin losses during frozen storage.

Loss (%) –18°C during storage for 12 months							
Products	**Vitamin C**	**Vitamin B1**	**Vitamin B2**	**Niacin**	**Vitamin B6**	**Panothenic acid**	**Carotene**
Beans (green)	52	0–32	0	0	0–21	53	0–23
Peas	11	0–16	0–8	0–8	7	29	0–4
Fruit*							
Mean	18	29	17	16	–	–	37
Range	0–50	0–66	0–67	0–33	–	–-	0–78

*Mean results from apples, apricots, blueberries, cherries, orange juice, juice concentrate (rediluted), peaches, raspberries, and strawberries; storage time not given. Source: Fellows [34].

Table 11.5 Vitamin losses during freeze-drying of vegetables and juice.

Food	**Loss (%)**			
	Ascorbic acid	**Vitamin A**	**Niacin**	**Pantothenic acid**
Green beans	26–60	0–24	10	–
Peas	8–30	5	0	10
Orange juice	3	3–5	–	–

Source: Fellows [34]

fruits and vegetables, as well as processed food. However, freeze-drying has less of an effect on the level of vitamin A and other vitamins.

Freeze-drying maintains optimal biological activity of the dried material and maximizes the true energy potential that can be attained from consumption of the nutritional product [43]. The retention of volatile aroma is also beneficial. With this mean of drying, aroma compounds are not released in the water vapour produced by sublimation but are captured in the food matrix [43].

11.2.3 Effects of Storage

A number of studies have been looking at changes in the bioactive components of food during storage. Okageri and Tasioula-Margari [44] studied the alteration of antioxidants, α-tocopherol and total phenols, during the storage of virgin olive oil exposed to light and in dark conditions and found that α-tocopherol decreased by 80% in 4 months under diffused light and at least 45% of phenol was decomposed in the same period of time. After storage in the dark conditions, the results showed that those two components were maintained at a higher level than storage under the light conditions. Likewise work done by Sharma and LeMaguer [38] indicated that when the fiber-rich fraction of tomato pulp was stored under three different conditions (vacuum and dark, dark and air, and air and light) at –20, 5 and 25°C for 60 days, the lycopene loss was maximum in the presence of air and light at 25°C.

Heat also generates compositional changes. Van der Merwe *et al.* [45] studied total tocopherols in palm-olein under heated storage. He found that after storage at 50°C for 52 weeks and during weekly determination of the total tocopherols of added copper to palm-olein samples, the tocopherol decreased sharply within the first 6 weeks, especially in the higher copper addition samples. Moreover, Kaul and Saini [46] worked with Kagzi lime juice and showed that in heat processed juice stored for 6 months at 12–40°C, the ascorbic acid content declined in about one half of the initial sample and more than 50% reduction occurred in heat pasteurized (90°C, 3 sec) samples. However, the study showed that preserving the pasteurized sample with SO_2 (700 ppm) can maintain the level of ascorbic acid.

Another factor which has an impact on bioactive deterioration is temperature. Fish and Davis [47] investigated the effect of cold storage condition on the decline of lycopene in watermelon tissue. The

data showed that after storage at –80°C for over one year, the lycopene content was more stable than when stored at –20°C. The same experiment also showed that the lycopene content decreased with temperature. Zafrilla *et al.* [48] showed that in ecological and conventional wine stored in a dark and cool place for over 7 months, anthocyanin content dramatically decreased 88% in conventional wine and 91% in ecological wine. Patil [49] indicated that in citrus beverages stored at 4°C for six weeks in different kinds of containers, total flavone and lycopene levels were significantly higher at the sixth week compared to initial levels followed by a dramatic decrease. Moreover, he suggested that the type of container used to store the juice influences the concentration of the functional components. Vitamin C loss in a plastic bottle container was much greater than for juice stored in a can.

11.3 Methods for the Determination of Antioxidants

11.3.1 Measuring Antioxidant Activity

The methods for determining antioxidant activity, generally evaluate this indirectly by measuring the change rate of any of theses intermediates viz., substrate, oxidant, initiator, and final products [5]. Antolovich *et al.* [5] indicated that the term "activity" needs to be defined as it applies to antioxidants. Relevant categories are:

- mechanistic intervention, e.g., free radical scavenger, catalytic decomposition;
- pro-oxidant suppression;
- rate of scavenging, e.g., near-diffusion or controlled; medium or substrate selectivity (e.g., aqueous, surface or liquid phase);
- concentration effectiveness (moles of free radicals scavenged per mole of antioxidant; and
- synergistic effect for other antioxidants.

11.3.2 Radical–Scavenging Methods

The hydroxyl radical and its subsequent radicals are stated to be one of the very harmful reactive oxygen species (ROS). In general,

they cause the oxidative injury of biomolecules [50]. For *in vivo* systems, hydrogen peroxide and superoxide molecules cannot solely oxidize lipids, nucleic acid and sugar unless they are produced as $OH^{\bullet}$ via Fenton reaction and/or iron-catalyzed Haber–Weiss reaction. As the idea of ROS has been defined, strategies for measuring the antioxidant activity and the ability to scavenge free radical are developed. The approach is to study the generation of free radical species including hydroxyl radical, superoxide radical or nitric radical and follow with the addition of antioxidants and measurement of their inhibition [5].

The examples of these methods include the use of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis (3-ethylbenzthiazoline-sulphonic acid) (ABTS) radicals. After allowing the DPPH radicals, for instance, to react with antioxidant (AH), monitoring the reduction of AH by using absorbance at 515 nm is used to measure the ability of antioxidant.

- *Diphenylpicrylhydrazyl (DPPH) radical*
  The synthetic radical (DPPH) is used to determine antioxidant activity. As the radical absorbs the light at 517 nm, antioxidant activity can be detected by monitoring the decrease in its absorbance. This method can be easily explained by the following reaction:
  $DPPH^{\circ} + AH \longrightarrow DPPH\text{-}H + A^{\circ}$ (Frankel [35])
  The parameter "antiradical efficiency" is evaluated due to the amount of antioxidant required for 50% decrease in initial DPPH radical concentration and the time taken to be stable at the 50% concentration (Antolovich *et al.* [5]). However, this method is rather inaccurate because the DPPH radical also reacts with other radicals such as alkyl [20]. Antolovich *et al.* [5] believed that the antioxidant effectiveness in foods must be evaluated by other methods because their activities in foods are reliant on a variety of factors including polarity, solubility and metal–chelating activity.
- *Total radical- trapping antioxidant parameter (TRAP) assay*
  To determine the total antioxidant activity of plasma or serum, the TRAP assay has been used. The measurement uses a peroxyl radical which is generated by 2,2′-azobis (2-amidinopropane) hydrochloride

(ABAP) to oxidize the antioxidant and the oxidation is measured by oxygen consumption [20]. Moreover, the induction period is standardized by addition of Trolox (a vitamin E analogue) as a reference water-soluble antioxidant. However, the use of the thermostated oxygen electrode cell in this method may generate a problem because the electrode will not maintain its stability over the period of time required [5].

11.3.3 Methods for Measuring the Oxidation of an Oil or Food Sample

In order to study the antioxidant effectiveness as well as antioxidant activity in oil and food sample, experiments need to be carried out under controlled conditions. Some of the methods are discussed below.

- *Sensory analysis*
  Trained panelists have to have an individual sensitivity to oxidative off-flavour. However, this method is unreliable because different individuals vary in their sensitivity to these off-flavours and their sense of taste and smell may vary depending on the stage of health [5]. Generally, sensory analysis may be useful when other chemical methods are not successful.
- *Head space analysis*
  As the volatile lipid decomposition is easy to detect by a consumer from oil products as off-flavours, the volatiles are trapped and then separated and identified by gas chromatography method. This method is used a lot for determining the oxidation in milk and milk products. Volatile free fatty acids, volatile carbonyls (methyl ketones and aldehydesI are determined in butter and Ghee products [51].
- *Peroxide value (PV)*
  Total hydroperoxide and peroxide oxygen content of lipids or lipid-containing materials are parameters representing peroxide value. In this method, potassium iodide is oxidized by hydroperoxides or peroxides to iodine which is then titrated with sodium thiosulphate solution, and starch is used as end-point indicator.

$$ROOH + 2H^+ + 2I^- \longrightarrow I_2 + ROH + H_2O$$

$$ROOR + 2H^+ + 2I^- \longrightarrow I_2 + 2\,ROH$$

$$I_2 + 2S_2O_3^{2-} \longrightarrow S_4O_6^{2-} + 2I^-$$

However this method is considered to have poor sensitivity and selectivity. The possible addition with iodine across the unsaturated bonds leads to low results, oxidation of iodine by dissolved oxygen and variations in reactivity of different peroxides [5].

- *Conjugated dienes*
  Since hydroperoxides are formed during the process of oxidation, the absorption of UV radiation at 233–234 nm can be detected due to the conjugation of the pentadiene structure. This mechanism is very useful because diene conjugation measures an early stage in oxidation. However, some products which are formed following hydroperoxide decomposition such as 9-hydroxyoctadeca-10, 12-dienoic acid and 13-hydroxyoctadeca-9, 11-dienoic acid can interfere with the absorbance. Tissue and body fluids also contain interfering substances such as haem proteins, chlorophylls, purines and pyrimidines. Thus, extraction of lipids into organic solvent is currently used to remove these substances [5].
- *Thiobabituric acid reactive substance (TBARS)*
  Thiobarbituric (TBA) is used to react with malonaldehyde which is a product of lipid oxidation. This results in a red condensation product (TBARS) that can be measured at 532–535 nm [52]. However, substances such as 2,4 alkadienal may also react with TBA and give a strong absorption at 532 nm. Moreover, other food components such as protein, and Maillard browning products can also interfere with the result.
- *Measurement of hexanal and related end-products*
  The primary products of oxidation such as hydroperoxides continue to decompose and form secondary products including epoxides, ketones (e.g., butanone, pentanones, octanones), hydrocarbon and saturated and unsaturated aldehydes such as hexanal. These

secondary mixtures are used to measure oxidation activity. A study of a ready-to-eat oat cereal has shown that when 5–10 μg/g of hexanal was detected, the rancid odor is noticeable.

- *The β-carotene bleaching method*
  The antioxidant activity is measured by the reduction in the yellow color of β-carotene which can be absorbed at 470 nm. The BCBT method is sensitive due to the strong absorption of β-carotene. The basic principle of this method is to measure the ability of a compound in order to minimize the loss of β-carotene during the oxidation of linoleic acid coupled with β-carotene in an emulsified aqueous system [53]. However, this method is unreliable because of its nonspecificity; its reaction to interference from oxidizing and reducing agents in crude extracts and linoleic acid does not represent typical food lipids [20].
- *Methyl linoleate oxidation (MeLo) method*
  The disappearance of methyl linoleate due to oxidation reactions and the formation of primary and secondary products of lipid peroxidation after incubation are detected. The antioxidant is added to the material extract at 40°C under dark conditions and the antioxidant activity was calculated as percent inhibition of hydroperoxide formation [54]. The formation of hydroperoxide can be determined until reaching the linear production (about 400 to 800 mmol/kg of MeLo) [54]. As this method needs to use the same amount of antioxidant, the problem is that it might limit or prevent the comparison of antioxidants in the same or different experiments. Devalos *et al.* [54] have developed a solution by using different antioxidant concentrations and expressing the result as 50% inhibition of hydroperoxide formation.
- *Oxygen radical absorbance capacity assay (ORAC-Fluorescein)*
  This method is based on the detection of chemical damage to β-phycoerythrins (β-PE), proteins isolated from *Porphyridium cruentum*, against peroxyl radicals, induced by 2,2′-(azobis(2-methyllpropionamide) dihydrochloride (AAPH), or hydroxyl radicals, generated

at copper-binding sites on macromolecules in the presence of ascorbate and Cu^{2+} [54, 55]. The protective effect of an antioxidant is quantified by assessing the area under the fluorescence decay curve (AUC) of the sample as compared to that of the blank in which no antioxidant is present. This method is thought to be one of the few methods that combines both inhibition percentage and inhibition time of the reactive species action by antioxidants into a single quality. Nonetheless, there are limitations in using β-PE as the probe. First, the inconsistency of β-PE from lot-to-lot may result in variable reactivity to peroxyl radical. Second, β-PE probe can be photobleached after exposure to excitation light at a certain time. Third, there was evidence that showed β-PE interacted with polyphenols due to the non-specific protein binding. Therefore, Ou *et al.* [56] developed the use of fluorescein (FL) (3,6,-dihydroxy-spiro[isobenzofuran-1[3H],9′[9H]-xanthen]-3-one) to replace β-PE. It was found that FL in comparison to PE does not interact with antioxidants [54, 57, 56].

11.3.4 Techniques Involving Bioactive Compound Determination

A number of studies have investigated the bioactive compounds in plant foods, including vegetables and fruits [58–60]. These studies used two conventional techniques, high performance liquid chromatography and ultraviolet/visible (UV-vis) spectrophotometry, as they are regarded as standard methods for bioactive compounds characterization and qualification such as anthocyanins [61].

11.3.4.1 High Performance Liquid Chromatography (HPLC)

HPLC is widely used to separate non-volatile molecules on the basis of molecular weight. HPLC can be broadly categorized as gel-permeation, adsorption (normal phase or liquid-solid), partition (liquid-liquid), ion-exchange and reversed-phase (RP) chromatography [62, 63]. HPLC with reverse-phase mode has often been used in the analysis of bioactive compounds in food and plant products such as vitamin C [64].

The reverse-phase mode consists of a polar elute which is usually water, containing a proportion of organic modifier such as methanol, and a non-polar stationary phase which is alkyl moiety chemically bound to a silica support materials [63]. An example for the use of HPLC with reverse-phase mode is the determination of ascorbic acid in orange juice, tomato juice, etc. A C_{18} column eluted with mobile phase with ion-pairing agents was used. The total ascorbic acid was detected at UV range from 210–254 [64].

Recently, for the identification of the bioactive compounds in plants extract, HPLC with mass scan spectrometry (MS) has been developed [65]. Mass spectrometry-based technique has been developed primarily in a medical chemistry environment, e.g., for drug indentification [66, 67]. This technique is used for molecular weight determination and as a special tool for complex structure identification problems. Lee and Kerns [67] explained the application of this method by ionization. After a molecule is ionized, the mass spectrometer provided molecular ions and weight of the separated compounds, and thus these masses are assigned to corresponding substructure of the compounds.

11.3.4.2 Spectrophotometry

Spectrophotometry can be described as the analysis of how a sample is affected by light. Light is a kind of wave which contains an electric component and a magnetic component. The two are perpendicular to each other (Figure 11.6).

When a light wave runs into a particle, or molecule, it can be scattered or absorbed. The molecule or substance that absorbs light is called a chromophore. Chromophores exhibit unique absorption spectra and can be defined by a wavelength of maximum

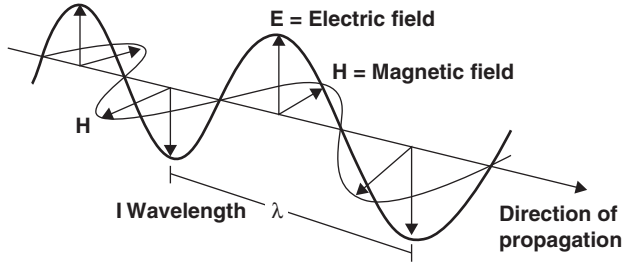

Figure 11.6 The electric component and magnetic component of light. Source: http://lf3.cuni.cz/chemie

absorption, or λmax. Most of the biological molecules absorb light in the visible and ultraviolet (UV) range. Absorption can be explained by the Beer Lambert law:

$$A = -\log (I/I_0) = \varepsilon\, d\, c$$

Where I_0 = initial light intensity, I = final light intensity, ε = molar extinction coefficient, d = thickness, and c = molar concentration.

From the equation, the amount of light absorbed relies on the nature of the chromophore, the concentration of the chromophore, the thickness of the sample, and other conditions such as pH. The concentration of substances can be determined when the molar extinction coefficient is known. The concentration of only some pigments can be measured by spectrophotometry. Anthocyanin, for instance, changes hue and intensity with pH. At pH 1.0, it exists as a purple color, the oxonium or flavylium form, but at pH 4.5 it is present as the colorless carbinol form. Thus, the difference in absorbance at 510 nm is proportional to anthocyanin content [68].

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12

Recent Advances in Storage Technologies for Fresh Fruits

Sukhvinder P. Singh[1,*] and Leon A. Terry[2]

[1]National Agri-Food Biotechnology Institute (NABI), Punjab, India
[2]Plant Science Laboratory, Cranfield University, Bedfordshire, United Kingdom

Abstract

Recent advances in storage technologies have impacted the postharvest industry worldwide. The introduction of 1-methylcyclopropene (1-MCP) based technology has given several benefits to the apple industry. The scope of application of 1-MCP in other fruits is increasing as the registration of this compound for edible horticultural commodities has already been made in many countries and is imminent in several others. The combination of 1-MCP with controlled/modified atmospheres with static O_2 and dynamic controlled atmosphere (DCA) is a promising hybrid technology that can improve the storage stability of fruits. The ultra-low oxygen (ULO) and DCA storage systems offer additional advantages in terms of maintaining fruit quality. The application of ULO, DCA, and 1-MCP has been mainly focused on apple fruit. However, there is a huge scope for extending their applications in other fruits. It is also probable that ethylene inhibiting/suppressing technologies other than cyclopropenes will also contribute to extending storage and shelf life. The bulk modified atmosphere packaging (MAP) and pallet covers are well integrated into the supply chain of soft fruits such as strawberries. These technologies are simple, cost-effective and pose minimal operational difficulties. The new fumigants such as nitric oxide are still at experimental stage and may find application in the near future. The choice and adoption of a storage technology or diagnostic device (e.g. biosensor) for a particular fruit is strongly influenced by the return on investment factor in addition to sustainability issues. This chapter reviews the recent advances in storage technologies for fresh fruits.

Keywords: CA storage, 1-MCP, ULO, DCA, nitric oxide, MAP

**Corresponding author*: spsingh@nabi.res.in

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12.1 Introduction

Fruits are an essential constituent of the human diet as they are rich sources of vitamins, minerals, bioactive compounds, and dietary fibre. Scientific evidence is increasing in favour of roles of phytochemicals in preventing and controlling several chronic diseases and lifestyle disorders. Diverse fruits are produced and consumed in different parts of the world. However, the consumption of fruits is inadequate in most countries. Public health policies are these days aimed at promoting the consumption of fruits in order to reduce the preventable burden of diseases. The difference between recommended and actual intake of fruits in developing countries is much higher than for the developed world.

Most fruits are perennial and produced seasonally. The narrow harvest window for many fruits causes wastage, market gluts, lowers returns to fruit growers, shortens the period of fruit availability to the consumers and puts seasonal pressure on processing industries. The storage of fruits is therefore indispensable to address these issues. Globalisation has given tremendous impetus to the international fruit trade, facilitating commercial movement of fruits from one part of the world to the other. This ensures availability of all types of fruits to the consumer throughout the year. The counter-season production in the northern and southern hemispheres is another advantage in the fresh fruit trade. Transport is a major activity in the fruit supply chain where storage technologies can be employed to preserve fruit quality to meet expectations of shippers, retailers, and consumers. The typical shipping time for fruits depends on the mode of transport and it varies from hours for the air transport to weeks for the marine shipment.

All fruits are living entities and continue to respire, transpire and sustain metabolic processes even after harvest. The physiological behaviour of fresh fruits is species or rather cultivar specific. The most common practice to regulate the physiological processes and biochemical changes in fruits is through postharvest temperature management. The maintenance of low temperature during storage and transport has been in place for several decades. However, with the advancement in the understanding of postharvest physiology and development of new techniques and tools, the storage potential of fruits has been enhanced.

12.2 1-Methylcyclopropene (1-MCP) Based Storage Technology

Ethylene is the gaseous ripening hormone which plays a regulatory role in determining the postharvest life of many fresh fruits. Depending upon the physiological behaviour, fruits produce ethylene during the process of ripening and also respond to exposure or presence of exogenous ethylene. Generally, the presence of ethylene at very small concentration in the storage atmosphere is capable of inducing and/or enhancing the process of fruit ripening and senescence leading to limited shelf life. The sources of ethylene, other than fresh produce, in the horticultural handling and storage facilities include the incomplete combustion of fuels in automobiles, forklifts and equipment powered by internal combustion engines. The cross contamination of ethylene can also occur due to ethylene produced by other commodities in the same storage chamber. Ethylene management is crucial to harness the full storage potential and maintain fruit quality. The removal of ethylene and/or inhibition of its action in stored environments are fundamental to maintaining the postharvest quality of most climacteric produce. In recent years, however, there has been a paucity of research on developing new and more efficacious ethylene scrubbing materials. In contrast, there has been an exponential increase in research using the ethylene binding inhibitor 1-MCP. Among different strategies to control ethylene, the postharvest exposure of fresh fruits to 1-MCP has emerged as the greatest tool of commercial importance [1, 2]. The discovery and commercial application of 1-MCP has revolutionized many postharvest industries across the world [3].

1-MCP is a gaseous ethylene action inhibitor, which is thought to bind irreversibly to ethylene receptors [4] and thereby prevent ethylene-dependent responses. The affinity of 1-MCP for the receptor is approximately 10 times greater than that of ethylene although there is debate on whether 1-MCP binds similarly to all ethylene binding sites [5]. Ethylene biosynthesis is also influenced by 1-MCP in some species through feedback inhibition [1]. 1-MCP has been used as a supplement to molecular approaches for identifying and understanding the spectrum of senescence and ripening processes under the direct control of ethylene perception. The gas has nontoxic mode of action, negligible residue and is effective at very low concentration (usually ≤ 0.6 ppm).

The major beneficial effects of 1-MCP in fresh fruits include suppression of ethylene production and respiration rates resulting in delayed fruit ripening and senescence, retardation of changes in fruit softening and skin colour, and alleviation of certain physiological disorders such as chilling injury and superficial scald. The benefits of 1-MCP in a range of fruits, vegetables and ornamentals have been reviewed elsewhere [1–3]. The information on the progress of literature on the physiological and biochemical effects of 1-MCP in fresh produce is available from the website (http://www.hort.cornell.edu/mcp/) updated by Professors Chris Watkins and William Miller at Cornell University.

The formulation of 1-MCP as a stable powder is reliant on it being complexed with γ-cyclodextrin. 1-MCP gas is easily released when the powder is dissolved in water. In 1999, the Environmental Protection Agency (EPA) approved the use of 1-MCP on ornamentals, marketed as EthylBloc® by Floralife, Inc. (Waterboro, SC, USA). SmartFresh™ as the trade name was subsequently developed by AgroFresh, Inc., a subsidiary of Rohm and Haas (Springhouse, PA, USA) for edible horticultural products. 1-MCP is registered for application in several fruits, especially climacteric ones, in many countries. The registration of 1-MCP is commodity and country specific. 1-MCP is internationally used in the apple industry to improve retention of textural and taste attributes of fruit during long-term cold storage. The application of 1-MCP in other fruits is also expanding. The beneficial or detrimental effects of 1-MCP in major fruit crops are described in Table 12.1.

12.3 Palladium Based Ethylene Adsorbers

Despite various ethylene scrubbing technologies being available (e.g. high temperature catalytic degradation, activated carbon, etc.) most commercial ethylene control systems rely on both adequate ventilation (often periodic) and oxidation of ethylene using potassium permanganate. Ventilation, however, is not appropriate in sealed environments (e.g. controlled atmosphere or some packaging formats) or where precise ethylene control is required. Potassium permanganate supported on activated alumina spheres has limited long-term efficacy in environments with high relative humidity (e.g. cold stores) such that more effective ethylene scavenging materials are needed.

Table 12.1 Summary of the effects of 1-MCP on retention of fruit quality and alleviation of physiological disorders in some fresh fruits.

S. No.	Fruit	Beneficial effects of 1-MCP	Detrimental effects of 1-MCP	Reference(s)
1.	Apple	Retention of fruit texture, reduction in physiological disorders (senescent breakdown, core browning, coreline browning, watercore and superficial scald)	Susceptibility to external CO_2 injury increases in 1-MCP-treated fruit.	[41–44]
2.	Apricot	Delayed fruit softening, reduced respiration and ethylene production rates	Reduced the evolution of aroma volatiles	[45]
3.	Avocado	Delayed fruit ripening, reduced flesh browning during cold storage		[46, 47]
4.	Banana	Delayed skin colouration and ethylene production	Uneven skin colouration, response is highly maturity dependent.	[48, 49]
5.	Citrus	Inhibition of rind pitting, inhibition of decay at low concentration		[50]
6.	Guava	Reduction in chilling injury and decay, retardation of fruit softening	Response may vary among climacteric and suppressed-climacteric cultivars	[51]
7.	Kiwifruit	Retention of flesh firmness, reduction in ethylene production and respiration rate		[52]

(*Continued*)

Table 12.1 (*cont.*)

S. No.	Fruit	Beneficial effects of 1-MCP	Detrimental effects of 1-MCP	Reference(s)
8.	Litchi	Delayed pericarp browning		[53]
9.	Mango	Inhibited fruit softening, increased shelf-life	Increased stem-end rot	[54, 55]
10.	Nectarine	Inhibited fruit softening	Increase in chilling injury, flesh browning, woolliness and flesh reddening	[56, 57]
11.	Papaya	Inhibition of fruit softening, delayed fruit ripening, if 1-MCP is applied at >25% skin colouration stage.	Failure to ripen, leathery and rubbery fruit, if 1-MCP is applied at <25% skin colouration stage	[58]
12.	Peach	Retention of flesh firmness and acidity	Chilling injury symptoms such as flesh browning, woolliness and flesh reddening are aggravated	[57, 59]
13.	Pear	Retention of fruit texture, reduction in superficial scald, senescent break-down, core browning, and watercore	Scald appears when ripening is resumed	[60, 61]
14.	Pineapple	Reduction in chilling injury		[62]
15.	Plum	Retardation of fruit softening, alle-viation of chilling injury symptoms. 1-MCP in combination with CA and MAP is more effective than alone.		[63, 64]

A palladium (Pd)-promoted powdered material that has significant ethylene adsorption capacity (4162 μL g^{-1} material) at 20°C and approximately 100% RH was identified and was shown to be superior to potassium permanganate-based scavengers when used in low amounts and in conditions of high relative humidity [6]. The material tested was developed by Johnson Matthey Plc and consisted of a Pd-impregnated zeolite giving finely dispersed particles [7]. Research at Cranfield University demonstrated that the Pd-promoted material at either 0.01 or 0.03 g L^{-1} effectively scavenged both exogenously administered (100 μL L^{-1}) and/or endogenously produced ethylene by banana or avocado, respectively, to sub-μL L^{-1} concentrations within a 24 h period. Optimum ethylene adsorption capacity was calculated as approximately 10,000 μL g^{-1}. Accordingly, corresponding inhibition of ethylene-induced ripening was observed. When removed, Pd-material did not disrupt subsequent ripening. More recent work has shown that it can extend the postharvest life of avocado fruit under both laboratory and real-world conditions [8, 9].

12.4 Ultra Low Oxygen (ULO) Storage Technology

Controlled atmosphere (CA) storage is one of the most successful innovations in storage technology during the last century. CA involves the precise modification of O_2 and CO_2 concentrations in the storage atmospheres to retard fruit metabolism, resulting in extended storage life and better fruit quality. Various researchers and technologists have endeavoured to reduce the concentration of O_2 to the lowest possible levels and enhance the concentration of CO_2 to the highest level so that the maximum benefits from inhibition of fruit metabolism are gained with uncompromised fruit quality. The recommended optimum CA conditions vary with commodity and/or cultivar. CA storage has been mostly used for apples and pears worldwide. The list of fruits being stored and/or transported under CA is expanding. ULO is a modification of CA in which O_2 concentration is maintained between 0.8 kPa and 1.2 kPa [10]. ULO has been adopted by the apple industry worldwide.

The beneficial effects of ULO have also been shown in other commodities such as grapefruit, kiwifruit, nectarine and pear [11]. The structural requirements for storage under ULO are stricter

than normal CA since the rooms need to be perfectly airtight. The monitoring of concentrations of O_2 and CO_2 is also more frequent to ensure the maintenance of high precision within a 0.1 kPa resolution [12]. The ULO-stored apples retain fruit quality in terms of firmness, skin colour, soluble solids and acidity longer than for the conventional CA system; however the aroma volatiles production is lower in the former [13, 14]. The scope of ULO can be extended to achieve other associated benefits with exposure to low O_2 atmospheres. The low O_2 atmospheres have potential fungistatic and insecticidal effects which can be further exploited to develop postharvest phytosanitary treatments for fruits. However, the application of insecticidal controlled atmospheres has not been accepted by regulatory authorities at this stage. The application of ULO for storage and/or transportation of fruits may expand in the near future.

12.5 Dynamic Controlled Atmosphere (DCA) Storage Technology

The static CA conditions optimised for a particular commodity/cultivar may not be always optimal depending upon the physiological conditions of the fruit. Therefore, the concept of dynamic controlled atmosphere (DCA) was evolved and researched extensively and later transformed into a commercial technology. DCA involves the monitoring of fruit responses to low oxygen in the storage atmosphere. The metabolic responses can be measured directly or indirectly such as respiration rate, ethylene production, ethanol concentration, or chlorophyll fluorescence. HarvestWatch™ is a fluorescence-based DCA technology, which has been developed by Canadian researchers in Nova Scotia, Canada. Chlorophyll fluorescence is influenced by low O_2 and high CO_2 and is an indirect measure of stress in apple fruit [15]. Unlike other stress indicators such as ethanol content, fluorescence measurement is a non-destructive, reliable and continuous approach to monitor the stress levels from a distance without removing the commodity from the storage system. HarvestWatch monitors the changes in fluorescence in response to low oxygen stress in fruit during storage [16]. The monitors (fluorescence interactive response monitor) used by HarvestWatch are connected to a computer control system and the operator adjusts oxygen levels in response to any fluctuations in the fluorescence signals. A buffer of ~0.2% oxygen is recommended to be added to

the level at which fluorescence changes in order to provide a safety margin against potential injury caused by anaerobic conditions [17].

The storage performance of several apple cultivars was evaluated by DeLong *et al.* [16] in which a direct comparison of HarvestWatch (<1% oxygen) was made against the static CA at 1.5% oxygen. The apple cultivars such as 'Golden Delicious' and 'McIntosh' were slightly firmer under HarvestWatch whilst no significant differences were observed for other cultivars such as 'Delicious' and 'Honeycrisp'. There are reports indicating reduction in the incidence of superficial scald in some cultivars of apples stored under HarvestWatch [18], but no significant differences in senescent related disorders were reported [19]. Low-O_2 thresholds determined for some apple cultivars by the HarvestWatch system are presented in Table 12.2. The use of the HarvestWatch system is limited to very airtight rooms suitable for ULO storage. The precise control over gases and perfect monitoring systems are the essential requirements for supplementation of ULO with HarvestWatch. However, the additional advantages of using this technology may not be significant if the risk assessment is conducted for storing the fruit at very close to anaerobic threshold concentrations of O_2. The commercial application of DCA was first taken up in Italy in 2004–2005 [12]. The authors have reported subsequent increase in

Table 12.2 Low-O_2 thresholds determined for each cultivar by HarvestWatch and the subsequent range of O_2 levels employed in dynamic CA storage [16].

Cultivar	Low-O_2 Threshold (kPa)	O_2 setting for each cultivar (kPa)
Cortland	0.5	0.6–0.8[a]
Delicious	0.4	0.5–0.8
Golden Delicious	0.5	0.5–0.8
Honeycrisp	0.4	0.5–0.8
Jonagold	0.5	0.5–0.8
McIntosh	0.8	0.9–1.0

[a] O_2 levels reflect the ideal setting (0.1–0.2 kPa above the detected low-O_2 threshold value) and the system variation encountered during the storage period.

the commercialization of this technology in the USA and Germany. The success of this technology will be determined by the return on investment factor by risk-benefit analysis.

12.6 Microcontrolled Atmosphere (MCA) and Bulk Modified Atmosphere Packaging (MAP) Technologies

Palistore™ is a microcontrolled atmosphere (MCA) technology for storage of pallets of fresh produce under CA and MA (Storage Control Systems, Inc., USA http://www.storagecontrol.com). This involves the use of a simple bag over the produce pallet to extend the storage life by modifying the atmosphere only inside each individual pallet. There is an aluminium pan which seals the bottom of the pallet, a cover fits over the stack of produce, and the bag is taped to the aluminium pan. The sealed pallets are stored in the normal air in a cold room until the product is to be marketed. The concentrations of O_2 and CO_2 are monitored via external tubing connected to the pallet using quick connect fittings which allow the tubes to stay in place when the pallet is removed from the cold store and are available for the next sealed lot of pallets. It can be controlled manually or by a complete computer control system that is capable of sampling up to 100 pallets. This method is very cost effective and suitable for various size growers.

Modified atmosphere packaging (MAP) is a simple and cost-effective technology in which the produce is enclosed in a polymeric film and the modified atmospheres are achieved either through produce respiration (passive MAP) or through gas flushing (active MAP). MAP in conjunction with recommended optimum storage temperature has a positive impact on fruit quality and extend the storage potential. The recent advances in polymer engineering and chemistry have introduced a variety of polymeric films having a range of permeability characteristics for water vapour and gases. The functionality of the polymeric films has also been improved by incorporating antimicrobial and antifogging properties. There are many success stories of MAP in fresh fruits, especially for small fruits such as strawberries, blueberries, raspberries, etc., however its use is still limited. The MAP of individual commodity in a plastic bag or the use of box liner has been practiced for over a long period. The use of bags and box liners are not suitable for commodities which require cooling after packaging. The application of

pallet covers is the right solution for achieving MA either passively or actively without any interference with cooling operation.

The plastic covers can be used for active/passive modification of atmospheres inside the pallet. For example, the pallet of strawberry cartons can be covered and the desired concentrations of gases can be flushed for active modification. The incidence of spoilage in soft fruits can be significantly reduced by high CO_2 atmospheres (ca. 15%). A recent study has compared the efficacies of different types of proprietary pallet covers in maintaining fruit quality of strawberry fruit during commercial shipment [20]. Strawberry fruit in vented plastic clamshells were palletized, forced-air cooled to 0.5–1.7°C and were covered with different cover systems (CO_2 West, PEAKfresh, PrimePro, and Tectrol). CO_2-releasing pads were placed inside the CO_2 West cover while Tectrol cover was sealed to the pallet base, a partial vacuum was applied, and pressurized CO_2 gas was injected inside. Other systems remained open at the base. Six separate shipments of palletized fruit were transported in refrigerated (0–3.9°C) truck trailers to distribution centres in either Florida or Georgia in 2.3–4.7 days. CO_2 concentrations within pallets at the beginning and end of transport were highest (11% to 16%) in the sealed Tectrol system and relatively low (0.06% to 0.30%) in the open CO_2 West, PEAKfresh, and PrimePro cover systems. The pallet covers reduced the weight loss by 38% to 52% during transportation compared to non-covered pallets. The fruit from the Tectrol pallets exhibited significantly less decay (36%) after 2-days shelf life than the CO_2 West (39%), noncovered control (41%), PrimePro (42%), and PEAKfresh (43%) pallets. These findings suggest that transporting strawberries in the sealed Tectrol pallet cover system, in which CO_2 concentrations were elevated to 11–16%, was most effective in complementing current low temperature management practices to maintain fruit quality. The pallet cover technology with immediate modification of atmospheres at the beginning offers several advantages in terms of maintaining fruit quality and controlling microbial spoilage during transport/storage, but may not be worthwhile for short transit times

12.7 Nitric Oxide Based Technology

Nitric oxide (NO) is a highly diffusible and reactive gas which makes it a versatile signal molecule capable of interacting with cellular targets via either redox or additive chemistry [21]. In plants,

NO plays a role in a broad spectrum of pathophysiological and developmental processes and is a ubiquitous molecule. NO has been known to play an important role in regulation of fruit ripening and senescence [22]. In fruits, its endogenous levels were reported to be higher in immature than in mature and ripe tissues of climacteric and non-climacteric fruits [23]. The endogenous levels of ethylene and NO during fruit development and maturation have inverse and stoichiometric relationships. NO levels decrease with maturation and senescence in horticultural crops [22, 23], thereby offering an opportunity for modulation of their levels with exogenous application to exert the opposite effect. Short-term exposure of intact and fresh-cut horticultural commodities to very low concentrations of NO retards their postharvest senescence [24–28].

Postharvest NO application in intact and fresh-cut produce delays ripening [29, 30], inhibits ethylene biosynthesis [22, 26, 27], prevents cut-surface browning [25, 30], and enhances resistance to postharvest diseases [27]. The mechanism of action of NO in delaying senescence of postharvest horticultural produce, though not completely understood, is via the inhibition of ethylene biosynthesis. The proposed mode of action through inhibition of ethylene biosynthesis is quite similar to 1-MCP, which blocks the perception of ethylene by the receptors. However, adequate evidence does not exist to ascertain the mode of action of NO. Postharvest NO treatment with very low concentration (10–20 ppm) has been found useful to retard the fruit ripening and maintain fruit quality during cold storage in kiwifruit, peach, plum, strawberry and tomato [31–34, 26, 28, 27]. Depending upon the concentration, NO could be either cytoprotective (low concentration) or cytotoxic (high concentration). Therefore, the optimum concentration of NO to achieve desirable results in specific produce still requires more work.

NO gas is most commonly obtained from a cylinder. But there are difficulties in the usage of NO gas for small-scale operations which comprise the majority of producers in most countries. Factors undermining the application of NO include the limited supply of NO cylinders in rural areas, occupational health and safety concerns arising from leakage and handling of high pressure systems, and difficulty in ensuring precise control for delivering small quantities of gas to small batches of produce [35]. The generation of NO gas *in situ* from a solid tablet would offer a more convenient and easier method for commercial use in the horticultural industry than release from a gas cylinder. Generation of NO can be

achieved through decomposition of many NO donor compounds including the diazeniumdiolates. Diethylenetriamine/nitric oxide (DETANO), the slowest-release NO donor among the diazeniumdiolates decomposes in acidic solution to generate two molar equivalents of NO with the reaction following first-order kinetics and being dependent on solution temperature, pH and the nucleophilic adduct. DETANO is highly soluble in water, easy to synthesise and is relatively stable as a solid compound and importantly, DETANO decomposition releases NO gas while other NO-donor compounds release NO in the form of NO^+ or NO^- [35]. Experimental evidence has been provided with the application of NO from gas cylinder as well as DETANO. However, the adoption of NO fumigation technology at commercial scale is still awaited.

12.8 Biosensors

Quality is a key attribute common to all horticultural products [36]. In virtually all cases, fresh produce quality is set at harvest and then inevitably declines during postharvest senescence. To evaluate quality, one must be able to measure quality-related attributes. Instrumental measurements are preferred to sensory evaluations in research and commercial situations as they reduce variations in judgment among different individuals. This approach can provide a means of transferring objective information on quality throughout the supply chain and is, thus, fundamental to ensuring greater vertical integration.

Fresh produce quality assessment can be either destructive or nondestructive. Currently, most nondestructive techniques are not yet appropriate for large-scale commercial use. For example, various methods using either chlorophyll fluorescence, delayed light emission, electronic nose technology, nuclear magnetic resonance imaging, optical tomography, ultrasound, and X-ray are either still in their infancy, or are still currently too expensive and/or unreliable to be adopted into most routine quality control (QC) operations. In the short to medium term, QC improvements for fresh produce may be based on established technology that is proven and inexpensive. Biosensors may offer one opportunity to fulfill this niche by enhancing the relevance and extent of QC tests being carried out through measuring specific target analytes that are directly related to produce quality [36].

The commercial application of biosensors has had a significant impact in a number of areas, particularly in the field of medical diagnostics. Disposable blood glucose biosensors, frequently used by diabetes sufferers to monitor their blood sugar levels, make up the vast majority of the current total biosensors market. Undoubtedly, this trend will continue yet opportunities to exploit biosensor technology in areas other than medical diagnostics do exist. One such industry where biosensor technology will be further exploited is in the food industry. Currently, however, food testing represents a very small percentage of the total market, but with advances in sensor longevity and stability and with new applications on the horizon, biosensors for food diagnostics are set to expand. Traditionally, the food industry has taken a very conservative approach to the introduction of biosensors but would benefit from improvements in QC, safety, and traceability that these relatively inexpensive devices can offer.

A biosensor can be defined as an integrated receptor transducer device, which is capable of providing selective quantitative or semiquantitative analytical information using a biological recognition element. Most biosensors which have been developed for fresh produce have been electrochemical in nature and mainly based on amperometry. In contrast to potentiometric biosensors, the operation of amperometric biosensors is defined by a constant potential applied between a working and reference electrode. The imposed potential encourages redox reactions to take place, causing a net current to flow. The magnitude of this current is proportional to the concentration of electroactive species present in solution.

Many amperometric biosensors to date have been based on the use of enzymes and it is evident that this format has been used for measuring quality-related analytes in various fresh produce types including onions, blackcurrant and strawberry fruit [36–40]. Typically, oxidase enzymes have been the most frequently exploited catalysts used for enzyme biosensor formats. In operation, amperometric biosensors tend to monitor either the oxygen consumed or the hydrogen peroxide generated. Both are electrochemically active; oxygen can be electrochemically reduced, and hydrogen peroxide can be oxidized. The current generated is proportional to the concentration of the enzyme substrate (i.e. the target analyte) present. Biosensor technologists have also adopted other approaches, including the use of mediators. These compounds are able to replace oxygen as an electron acceptor and to operate at a

much lower operating potential, reducing the effects of other electrochemically active species found in many food matrices [36]. To date, a few fresh produce biosensors have been developed and in some cases commercialised. Examples include pyruvate biosensors for measuring pungency in onions [37, 38], and glucose-oxidase-based biosensors for measuring glucose in strawberry and blackcurrant [39, 40]. Biosensors have also been produced which are capable of measuring antioxidant capacity and individual anthocyanins in berry fruit [40].

12.9 Conclusions

The postharvest handling and storage practices have continually evolved through the introduction of new technologies and methods, often while responding to various factors such as consumer choices, regulatory requirements, and market demands. The evolution of these technologies has enabled the industry to deliver high-quality produce for domestic and international markets and develop resilience to respond to varied and sometimes unpredictable challenges. Fresh fruits are highly perishable and require special postharvest care in terms of optimum storage environment to maintain their quality. Low temperature storage in combination with various chemical treatments and/or atmosphere modifications has been employed for long-term storage and transport of fresh fruits. The international trade of fruits has surged several folds in the past decade which necessitated the increase in storage and shelf life considering the changeability of global markets. With all quality concerns, the transcontinental shipment of fruits is a challenging task. The dimensions of assessment of fruit quality require restructuring as consumers are becoming more discerning. The storage potential of fresh fruits is generally determined following conventional approaches such as retention in firmness, acidity and skin colour. However, the storage potential of a commodity may be significantly lower than that claimed if flavour and nutritional quality parameters are considered. The demand for consistently high quality produce is the need of the hour.

Recent advances in storage technologies have impacted the postharvest industry worldwide. The introduction of 1-MCP-based technology has given several benefits to the apple industry. The scope of application of 1-MCP in other fruits is increasing as the

registration of this compound for edible horticultural commodities has been already made in many countries and is imminent in several others. The combination of 1-MCP with controlled/modified atmospheres with static O_2 and DCA is a promising hybrid technology that can improve the storage stability of fruits. ULO and DCA systems offer additional advantages in terms of maintaining fruit quality. The application of ULO, DCA, and 1-MCP has been mainly focused on apple fruit. However, there is a huge scope for extending their applications in other fruits. It is also probable that ethylene-inhibiting/suppressing technologies other than cyclopropenes will also contribute to extending storage and shelf life. The bulk MAP and pallet covers are well integrated into the supply chain of soft fruits such as strawberries. These technologies are simple, cost-effective and pose minimal operational difficulties. The new fumigants such as nitric oxide are still at experimental stage and may find application in the near future. The choice and adoption of a storage technology or diagnostic device (e.g. biosensor) for a particular fruit is strongly influenced by the return on investment factor in addition to sustainability issues. Researchers and industry have successfully confronted the challenges of the past and can capitalise on the opportunities that lie ahead, so that the fresh fruit industry continues to contribute to the economic and social wellbeing of growers and consumers for many decades to come.

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13

Ultrasound Applications in Food Technology: Equipment, Combined Processes and Effects on Safety and Quality Parameters

Rui M.S. Cruz[1,3,*], Igor Khmelinskii[2,3] and Margarida C. Vieira[1,3]

[1]Department of Food Engineering, Institute of Engineering, University of Algarve, Faro, Portugal
[2]Department of Chemistry and Pharmacy, Faculty of Science and Technology, University of Algarve, Faro, Portugal
[3]CIQA-Chemistry Research Centre of Algarve, Department of Chemistry and Pharmacy, Faculty of Science and Technology, University of Algarve, Faro, Portugal

Abstract

The need for more efficient processing technologies is a current research objective since consumers are increasingly looking for safe 'fresh-like' food products processed with minimal heat. The interest for non-thermal technologies has increased over the years since they may reduce the severity of the conventional thermal treatments and consequently contribute to the production of safe food products with higher nutritional and sensory quality. In the last fifteen years the application of ultrasound in food products has been a subject of research of several groups worldwide. This chapter discusses the application of ultrasound technology for improving processing efficiency, equipment design, its application in food preservation and its benefits and drawbacks in different quality attributes in a variety of food products and processes.

Keywords: Ultrasound, thermosonication, manosonication, manothermosonication, food quality, safety

**Corresponding author*: rcruz@ualg.pt

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13.1 Introduction

The history of power ultrasound can be traced back to the discovery of the piezoelectric effect by Pierre Curie in the late 1800s. He found that asymmetrical crystals such as quartz and Rochelle salt (sodium potassium tartrate tetrahydrate) generate an electric charge when mechanical pressure is applied. Conversely, mechanical vibrations are obtained by applying electrical oscillations to the same crystals. Practical implementation of piezoelectric crystals in ultrasonic transducers was a result of experiments conducted by Paul Langévin in 1915 [1–4].

The application of ultrasound at an industrial level is a rapid growth area in the field of ultrasonic engineering, encompassing food, pharmaceutical, petrochemical, and nuclear industries [5].

Ultrasound applications alone or in combination with other treatments have received great attention due to their potential to produce safe food products with higher nutritional and sensory quality. Work has been conducted in different food products and treatment combinations to confirm the clear benefits of the ultrasonic technologies and their main drawbacks. In terms of consumer acceptability, the application of emerging technologies such as ultrasound have raised much lower levels of concern than 'irradiation', 'genetic engineering,' and other more controversial technologies [6].

Ultrasound is a form of energy generated by sound waves equal or above 20000 vibrations per second (20000 Hz or 20 kHz), generally above frequencies that can be detected by the human ear, and are able to travel through gas, liquid and solid materials [7, 8].

Ultrasound can be classified into two different categories: low frequency and high power (frequency in the kHz range) and high frequency and low power (frequency in the MHz range) [9–11]. The low power ultrasound, typically less than 1 Wcm^{-2}, is commonly used in medicine for diagnostic purposes, and also used to evaluate texture, composition or viscosity of foods. The high power ultrasound (typically in the range 10–1000 Wcm^{-2}) has been used for many years to produce emulsions, disrupt cells and disperse aggregated materials [12].

High energy ultrasound effects on liquid systems are mainly related to the cavitation phenomenon. Ultrasound propagates via a series of compression and rarefaction waves induced on the molecules of the medium passed through [13]. At a power sufficiently high, the

rarefaction amplitude may exceed the attractive forces of the liquid molecules and cavitation bubbles form from gas nuclei existing within the fluid. These bubbles, distributed throughout the liquid, grow over a period of a few cycles to a critical size until they become unstable and violently collapse [14–16]. The implosion of cavitation bubbles leads to energy accumulations in hot spots, generating extreme temperatures (5000 K) and pressures (1000 atm), which produce, in turn, very high shear energy waves and turbulence in the cavitation zone [17, 18]. The combination of these factors (pressure, heat and turbulence) has a variety of effects on the ultrasound-irradiated system. A combination of several factors: energy (in kWhL) and intensity (in Wcm^2), along with the medium viscosity, surface tension, vapour pressure, nature and concentration of dissolved gas, presence of solid particles and temperature and pressure of the treatment, determine the extent of cavitation. Additionally, when liquid processing is intended to be scalable, ultrasonic density (Wcm^3) should also be considered, so that it takes into account the extremely different acoustic streams and the corresponding different results in the new volume [19].

Another phenomenon resulting from the bubble size variation and subsequent collapse is the development of strong microstreaming currents, associated with high-velocity gradients and shear stresses that change the media characteristics [17]. Moreover, part of the acoustic energy can be transformed into heat; however, depending on the operating conditions and substrate, the temperatures reached are usually lower than 70 °C [20]. Another important effect is the sonolysis of water molecules generating highly reactive free radicals (Figure 13.1), which may react and modify other molecules [21]. Different effects on physical, chemical and biochemical parameters can be obtained, depending on frequency and amplitude of the ultrasound application [22, 23].

This article reviews the application of ultrasound technology, addressing equipment design and its use as a treatment for

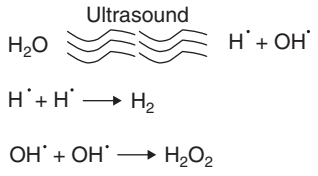

Figure 13.1 Water sonolysis and radicals combinations.

improving food processing efficiency, as well as its benefits and drawbacks, affecting several safety and quality parameters in a variety of food products and processes.

13.2 Equipment Design

Ultrasounds are produced by an electric apparatus, the generator, which can be controlled to create vibrations at a desired frequency. The ultrasound power supply converts the 50/60 Hz line voltage into high frequency electrical energy which is then transmitted to the piezoelectric transducer within the converter, and transformed into mechanical vibrations. These vibrations are intensified by a probe, which when inserted in a liquid medium creates pressure waves [24–28]. These transducers may be strategically placed on the sidewall or at the bottom of a tank or can be directly immersed in the liquid [29].

There are different designs of ultrasound devices, with several sizes and geometries, construction materials, output powers, frequencies and capacities. At a laboratory scale there are two common types of ultrasound equipment: the ultrasound probe or horn (Figure 13.2a) with a separate generator and the ultrasound bath/vessel (Figure 13.2b).

Most of the standard ultrasound baths operate at around 40 kHz and are of rather low power in order to avoid cavitation damage to the tank walls, and may have heating and a timer [30]. The bottom of the vessel is irradiated with single or multiple transducers and the active zone is restricted to a vertical plane just above the transducers with the maximum intensity at the centre of the

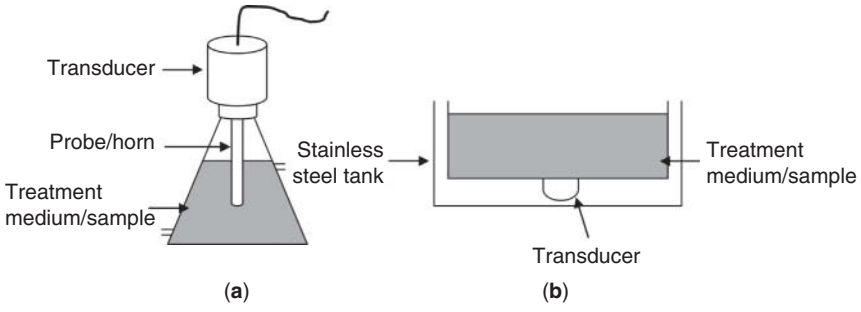

Figure 13.2 (a) ultrasound horn; (b) ultrasound bath.

transducer [31]. Thus, the area of the irradiating surface should be increased to the maximum possible value, so as to get better distribution/dissipation of energy in the vessel [32].

Despite several studies reporting the use of ultrasound baths with great advantages, some problems may occur during the samples processing. The treated material might agglomerate in certain regions within the bath so that only the outside of the material agglomeration is exposed to the ultrasound. Moreover, the material may also sink or float on the surface resulting in different outcomes for the same sample. These types of problems may be circumvented using mechanical stirring [33]. Ultrasonic intensity distribution inside the vessel is also heterogeneous. A simple and inexpensive method to detect high ultrasonic intensity zones is the aluminum foil test. The highest intensities inside the bath are located at the greatest damaged zones of the aluminum foil [34]. The active regions should be known and should be maximized for a uniform distribution of the ultrasonic power, allowing an effective utilization of transmitted acoustic energy to carry out given physicochemical transformations through the bubble oscillation activity. Once this information is known, the reaction mixtures can be kept at that particular location or the transducer arrangement can be optimized to maximize the overall volumetric efficiency of energy utilization [35]. Lerin *et al.* [36] reported the use of an ultrasonic unit with an ultrasonic transducer fitted at the bottom of the bath horizontally along the length of the bath. This setup offers much larger effective cavitational area compared with the conventional immersion-based axial transducers and hence results in a uniform distribution of cavitational activity in the ultrasonic bath.

The use of multiple sound sources operating at similar and/or different frequencies of irradiation and optimization of the power input to the systems helps in achieving uniform and more intense cavitating conditions as compared with the conventional designs [37]. In order to increase the existing active zones in the vessel, the position of the transducers (if multiple transducers are used to successfully operate at very high power and frequency) can be easily adjusted so that the wave patterns generated by the individual transducers overlap, resulting in uniform and increased cavitational activity. Different configurations are reported in the literature. Gogate *et al.* [38] developed a hexagonal vessel with each side of the hexagon hosting multiple transducers (3 in number per side) having equal power rating 150 W per side (total power

dissipation of 900 W when all the transducers with combination of 20+30+50 kHz frequencies are functional). The two opposite faces of the flow cell have the same irradiating frequency. The operating frequency of transducers presents values of 20, 30 and 50 kHz and can be operated in different combinations (7 in total) either individually or in combined mode. Later, Gogate and Pandit [39] indicated that the placement of transducers on parallel plates in a hexagonal configuration results in near-uniform distribution of the cavitational activity. Triangular pitch for positioning the transducers in the case of ultrasonic bath [40–41], tubular reactors with two ends either irradiated with transducers or one end with transducer and the other with a reflector [42], and parallel plate vessels with each plate irradiated with either the same or different frequencies [43, 44] are other types of configurations already studied. Thus, it is important to understand the dependence of the cavitational activity regarding the location of the transducers in the vessel/reaction medium, frequency of ultrasound, dimension of vessel, height of liquid medium in the vessel, power density and surface area of the irradiating element [29].

The other type of equipment known as ultrasound horn devices, are also commonly used. These are typically immersion-type transducers, which deliver high intensities in comparison to ultrasound vessels. However, some problems and heterogeneous outcomes may arise if the equipment setup and specifications are not taken into account. Thus, the horn selection must be appropriate for the volume of the sample to be treated and the ratio between the sample and the treatment medium optimized. On the other hand, the distance between the horn tip and the bottom of the vessel is also a parameter to be measured and standardized in order to avoid dead cavitation zones since its intensity decreases exponentially as one moves away from the horn and disappears at a distance of as low as 2–5 cm depending on the maximum power input of the equipment and also on the operating frequency [34, 45].

Horns are used in various shapes, sizes and different materials, according to the application, but like other components should be resonant at the operating frequency. Most of the horns are made of titanium or aluminium alloys, steel and stainless steel and may have different shapes such as cylindrical, tapered, exponential, stepped, full wave, half wave, half wave with an opening and half wave booster, dual probe, multi probe, cup horn and horn for vials (Figure 13.3). It is imperative that the horn has the required

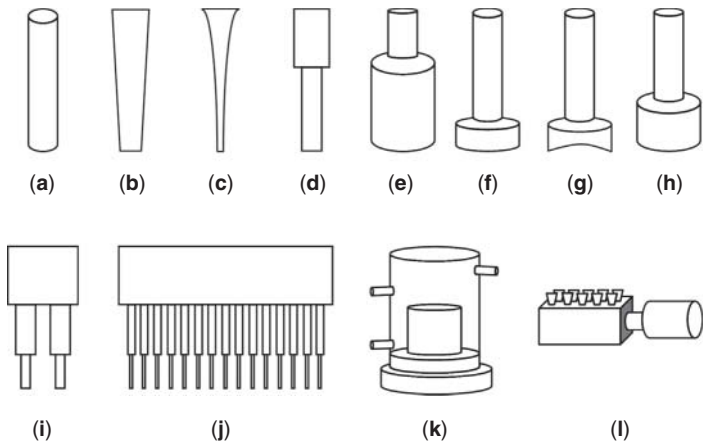

Figure 13.3 Horn shapes: a) cylindrical, b) conical, c) exponential, d) stepped, e) full wave, f) half wave, g) half wave with an opening, h) half wave booster, i) dual probe, j) multi probe, k) cup horn, and l) horn for vials.

dynamic properties, which must be already determined in the design phase [37].

Most of the reactions are not only influenced by the frequency and intensity of ultrasonic irradiations but also by the shape of the vessel/horn, operating power density, fraction of dissolved gases, physicochemical properties of liquid medium, surrounding pressure field in the vessel and operating temperature [29].

The temperature of the setup must be controlled since the ultrasound devices generate local heating. The ultrasound vessels normally have a temperature sensor. Nevertheless, if the sample is not stirred, different temperatures in diverse locations of the vessel may be obtained.

In the case of devices with ultrasound horns, a cooling vessel connected to a thermostatic bath to dissipate heat is commonly used for temperature control. However, depending on the setup of the apparatus and the objective of the study one or more factors must be controlled. In published work about ultrasounds there is a lack of detailed information about the equipment brand, frequency, amplitude and/or power, equipment setup and the sample treatment.

Although originally both types of equipment were used for cleaning, degassing, homogenizing and extraction, they are now commonly used for studies of improving the quality of food products or the efficiency of processing treatments. Furthermore,

several companies in the US and Europe such as Sonics and Materials, FFR Ultrasonics, Industrial Sonomechanics, Ultrasonic technique–INLAB, and Hielscher are supplying ultrasound equipment for industrial applications such as in-line ultrasound probes for liquids with power outputs of up to 16 kW, radial fluid processors (10–100 kW), ultrasonic knives and sets of ultrasound probes for multi-sample application (maximum output power of 750 W) for different batch sizes (up to 1 m^3) and flow rates (>10 m^3/h). Nevertheless, there is a limited number of processing applications being carried out on an industrial scale due to the lack of knowledge required for scaling up successful laboratory scale processes in diverse fields such as material science, acoustics, chemical engineering, etc. [29].

13.3 Ultrasound Application for Improving Processing Efficiency

As previously referred to, the functions of ultrasound laboratory devices were initially limited. Nevertheless, some of those were adapted according to their new function, helping to improve other types of processing operations such as brining, cutting, drying, emulsification, extraction, freezing, homogenizing, mass transfer, mixing and osmotic dehydration. Different methodologies, combined processes, their advantages and drawbacks will be discussed in this section.

Ultrasound as an emerging food preservation technique can also be used as a 'hurdle technology', in a sequence of mild (low intensity) treatments that inhibit or inactivate the factors responsible for food spoilage, avoiding the use of single treatments in more severe conditions [46]. Hurdles can be classified as physical (e.g. temperature, pressure, packaging), physicochemical (e.g. pH, a_w) or microbial (e.g. bacteriocins). The hurdle technology has already been successfully applied with traditional techniques of food preservation [7, 27, 47–49].

Ultrasound can be used alone (sonication) or combined with different processing treatments in order to increase their process efficiency and reduce the process severity by lowering the temperature [7]. Ultrasonication is, in many situations, combined with temperature (commonly referred as TS-thermosonication) [20, 50–53] pressure (MS-manosonication) [54] or heat and pressure

(MTS-manothermosonication) [55] resulting in different effects [25, 26, 56–65].

In some conditions, the combination of several processing techniques with ultrasound can be simply additive, but depending on the type of combination or kind of food product synergistic or antagonistic effects are possible [27]. Moreover, in some processing combinations and depending on the main goal, it is possible to have both antagonistic and synergistic effects.

Yildirim *et al.* [66] used ultrasonic tanks (25 kHz, 100 W; 40 kHz, 100 W and 25 kHz 300 W) at different temperatures (20–97 °C) in order to increase the water absorption of soaked chickpeas. One hundred grams of chickpea seeds were immersed in 2000 mL deionized water (1:20). It was concluded that water diffusion rates into chickpeas significantly increased with increasing of soaking time, temperature and power of ultrasound. However, at lower temperatures (20–40 °C) and high ultrasonic frequency (40 kHz) the water absorption rate was lower compared to the control. In other studies [67–69], where the objective was to cut samples efficiently, ultrasound equipment was adapted to cutting blades allowing, by vibration, the reduction of applied cutting force and cutting work. Moreover, these systems allow the cutting of fragile samples with high success rates. However, some of the problems regarding this type of system includes non-linear vibration of the blade and noise levels. The cutting conditions must be configured according to the type of blade, food product, shape and depth of cut [30]. Another type of application was reported by Tan *et al.* [70] in which a planetary mixer bowl was adapted to an ultrasound bath system in order to study its effect on a sponge cake quality. The ultrasound was able to enhance the mixing process by resulting in lower batter density and flow behaviour index, and higher overrun and viscosity compared with the non-aided mixing. With the 2.5 kW ultrasound-assisted mixing for entire batter mixing of 9 min, a cake with better quality was produced in terms of lower cake hardness, and higher cake springiness, cohesiveness and resilience. Delgado *et al.* [71] also reported the use of an ultrasound bath system (40 kHz and 131.3 W) to assist immersion freezing of fresh apple cylinders. It was concluded that the average freezing rate was significantly improved by up to 8% when ultrasound was applied from 0 °C or -1 °C for 120 s. A similar study was also conducted by Li and Sun [72] in which potato sticks were frozen in an ultrasonic bath (25 kHz and 7.34–25.89 W), and the freezing rate was also enhanced.

Mortazavi and Tabatabaie [73] also reported that ultrasound (20 kHz) was beneficial to ice cream freezing since it reduced process time and led to a product of better quality, reducing crystal size and preventing incrustation of the freezing surface.

In another work, Deng and Zhao [74] used an ultrasonic bath (50–55 kHz and 185 W) to improve osmotic dehydration of Fuji apples with high fructose corn syrup. Samples treated with ultrasound showed the lowest values of moisture content and water activity, presenting the highest values of hardness and crispness.

Oliveira *et al.* [75] also showed the advantages of ultrasound (25 kHz and 60 W) as a pre-treatment in the dehydration process of Malay apple. The results showed a reduction of about 27.3% in the total drying time. Gallego-Juarez *et al.* [76] reported that high intensity ultrasound (20 kHz) in combination with hot air systems (1.3–3 m/s and 50–115 °C) resulted in higher drying rates for carrot drying even at lower temperatures compared with the traditional drying system. The use of ultrasound (20–40 kHz and 0.5–43 W/cm^2) as a treatment prior to drying (0.3 m/s and 60 °C) of button mushrooms, Brussels sprouts, and cauliflower also reduced the drying time significantly [77]. Cárcel *et al.* [78] reported that drying (0.5–12 m/s and 50 °C) persimmon in ultrasound (21.8 kHz and 75 W) assisted drying was faster than the drying treatment without ultrasound. Cárcel *et al.* [79] reported that the application of high intensity ultrasound (0–33 kW/m^3) during olive leaf drying (1 m/s and 40 °C) reduced the antioxidant activity of extracts at the equilibrium but increased the initial extraction rates compared with conventional hot air drying.

Fernandez and Rodrigues [80] also reported that ultrasonic (25 kHz, 4870 W/m^2 and 30 °C) pretreatment of banana prior to drying (60 °C) reduced the overall drying time by 11%. Brncic *et al.* [81] studied the impact of high power ultrasound (24 kHz and 200 W) pre-treatment on drying rate and textural properties of infrared dried (85 °C) apple slices. The results showed that the use of different amplitudes of ultrasound reduced the time of drying and allowed the elimination of more water from the apple slices. The results also showed that hardness of samples gradually increased (50% amplitude-97.260 N; 100% of amplitude-217.90 N) with an increase of ultrasound intensity. Maskooki *et al.* [82] studied the effects of combined caustic soda and ultrasound (28 kHz, 150 W and 0–60 min) on reducing the drying time of grapes in raisin

production. The combined treatment with ultrasound and caustic soda significantly reduced the time required for dehydration.

Pohlman *et al.* [83] studied the effect of ultrasound (20 kHz, 1000 W at 62–70 °C) and convection cooking on beef longissimus and pectoralis muscles. The ultrasound treatment resulted in greater cooking speed, moisture retention and efficiency of energy consumption than convection cooking (required two to three times more energy to cook meat sections).

Several works [84–100] also report the positive effect of ultrasound in the extraction of valuable compounds, resulting in high quality products. This treatment, besides being a clean method, allows accelerating heat and mass transfer, thus increasing the extraction yields. The use of moderate temperatures is also another benefit, which is beneficial for heat-sensitive compounds. Moreover, it requires reduced working time and low investment; however, large amounts of solvents can be consumed.

In the extraction of sugar from beets the use of power ultrasound provided a greater penetration of the solvent into cellular matter and improved mass transfer. Ultrasound causing disruption of the biological cell walls enhanced the release of the internal contents [9]. Khan *et al.* [101] reported that ultrasound-assisted extraction (25 kHz, 150 W at 40 °C) proved to be more efficient for extraction of polyphenols from orange peel as compared to the conventional method (25 °C, 1:1 ethanol-water solution, stirring for 30 min). Recently, Horžic´ *et al.* [102] showed that a probe ultrasound-assisted extraction (20 kHz, 600 W for 3–30 min) was also more efficient for the extraction of total flavonoids compared with a conventional method (heated solvent: water-80 °C; 75% ethanol-boiling point for 3–30 min) and a bath ultrasound-assisted extraction (37 kHz, 200 W for 3–30 min).

Also, in the emulsification process, ultrasound has been used industrially in the manufacture of salad cream, tomato ketchup, peanut butter and some cream soups and fruit juices [9].

It was also concluded that ultrasound technology can be applied with success in the brewing process since nitrogen gas bubbling and ultrasound vibrations can decrease dissolved carbon dioxide in tanks and can help to control yeast metabolism, foam separation and height [103].

In a study in which ultrasound was applied to cheese brining, the rate of water removal and sodium chloride gain increased when ultrasound (30 kHz, 300 W at 5–20 °C) was applied [104]. For the

same degree of dehydration, the ultrasound treatment at 5 °C was 4 times faster than the control treatment. Lieu and Le [105] applied ultrasound (35 kHz, 60 W at 60–80 °C) in grape mash treatment in juice processing. In comparison with traditional enzymatic treatment, sonication treatment increased extraction yield by 3.4% and shortened the treatment time threefold.

The following sections will focus on the effects of ultrasound and combined treatments in food preservation and food quality attributes.

13.4 Food Preservation Applications

13.4.1 Enzymes

The inactivation of enzymes such as lipoxygenase, peroxidase, pectin methylesterase, polygalacturonase and polyphenoloxidase, which might contribute to a food product with undesired characteristics, is often one of the main objectives in food processing.

Ultrasound alone or combined with other preservation methods have been applied for enzyme inactivation in foodstuffs [23]. This inactivation is achieved, probably, due to cavitation [9, 106].

The cavitation may damage enzymes, probably by unfolding and scrambling the native protein and breaking the chain into radicals or smaller polypeptides [107]. Moreover, conformational changes in the enzyme tertiary structure, as well as in the active site three-dimensional structure, affect the enzyme-substrate interaction, leading in some cases to an optimal stage of consumption of the substrate or in other cases to a reduced stage of consumption [108]. The success of ultrasound in controlling enzymatic activity is mainly influenced by intrinsic and extrinsic factors such as type of enzyme and its concentration, temperature, pH and medium composition [109]. Table 13.1 presents the application of ultrasound and its effects on some relevant enzymes in several food products.

13.4.2 Microorganisms

Consumers' increased demand for food processing methods that have a reduced impact on nutritional content has stimulated the use of ultrasound, coupled with standard sterilization and pasteurization methods, for microbe inactivation [5].

Table 13.1 Ultrasound and the effects of combined treatments on some enzymes in several food products.

Food product	Conditions	Results	Reference
Apple	Sonication 40 kHz plus 1% ascorbic acid	Higher polyphenoloxidase and peroxidase inactivation	[110]
Barley	Thermosonication 20 kHz, 30–70 °C	Higher α-amylase inactivation	[111]
Lemon	Thermosonication 83%, 50 °C	Higher pectinesterase inactivation	[112]
Milk	Thermosonication 20 kHz, 150 W, 61–75.5 °C	Higher lactoperoxidase inactivation	[20]
Orange juice	Manothermosonication 20 kHz, 72 °C, 200 kPa	Higher pectin methylesterase inactivation	[25]
	Thermosonication 20 kHz, 72 °C	Higher pectin methylesterase inactivation	[27]
	Sonication 20 kHz 0.42–1.05 W/mL	Higher pectin methylesterase inactivation	[113]
Pineapple juice	Sonication 19 kHz 0.67–3.3 W/mL	Higher polyphenoloxidase inactivation	[114]
Seedless guava	Thermosonication 20 kHz, 80–95 °C	Higher peroxidase inactivation	[115]
Tomato	Ultrasound 23 kHz 15–75%	Higher peroxidase inactivation	[116]
	Thermosonication 20 kHz, 100 W, 50–72 °C	Higher pectin methylesterase inactivation	[28]

(*Continued*)

Table 13.1 (*cont.*)

Food product	Conditions	Results	Reference
Tomato juice	Manothermosonication 20 kHz, 196 kPa, 117 μm amplitude, 70 °C	Higher pectin methylesterase and polygalacturo-nase inactivation	[26]
	Thermosonication 24 kHz, 400 W, 60–65 °C	Higher pectin methylesterase inactivation	[117]
	Thermosonication 20 kHz, 50–75 °C	Higher pectin methylesterase and polygalacturonase inactivation	[118]
Watercress	Thermosonication 20 kHz, 125 W, 82.5–92.5 °C	Higher peroxidase inactivation	[50]

The effectiveness of ultrasound against microorganisms is dependent, like any other food preservation process, on the type of bacteria, the exposure/contact time, the composition of the food, and the treatment temperature. Ultrasound treatment has been reported to be appropriate to meet the FDA's requirement of 5-log reduction of foodborne pathogens in fruit juices [119]. The destruction of pathogens is thought to be due to the pressure changes caused by the ultrasonic waves [120, 121]. These micromechanical shocks disrupt cellular structural and functional components up to the point of cell lysis [7]. Ultrasound is also responsible for the production of localized heating and free radicals, contributing to the DNA damage [24]. The Gram-positive bacteria are known to be more resistant than gram-negative ones, probably due to their thicker cell wall which provides them a better protection against ultrasound effects. In terms of shape, cocci are more resistant than bacilli due to the relationship of cell surface and volume [122].

Recent transmission electron microscopy and flow cytometry studies of yeast, and Gram-negative and Gram-positive bacteria

have also demonstrated that (a) microbial cells contain several targets for the disruptive action of ultrasound, including at least the cell wall, the cytoplasmic membrane, the DNA, the internal cell structure, and the outer membrane; (b) cytoplasmic membranes do not appear to be the primary target of ultrasound for *Saccharomyces cerevisiae*, *Escherichia coli*, and *Lactobacillus* spp.; and (c) the primary target depends on the microorganism (for instance, the outer membrane in *E. coli*) [123, 124]. When ultrasound is combined with heat or pressure, the mechanical cell disruption is enhanced, thus several studies have focused on the effects of combined treatments. Cameron *et al.* [125] studied the destructive effect of cavitation on microbial cells by transmission electron microscopy techniques. The cavitational forces, induced by ultrasonication (20 kHz, 750 W at 24–26 °C) caused irreparable damage to the outer cell wall and inner cell membrane of the tested microorganisms. In another study the application of sonication (160 kHz and 100 W) in peptone water during 10 min, resulted in a 4-log reduction in *Salmonella* spp. viable cell count [126]. Also, in a study on lettuce [127], the ultrasound application (25–70 kHz, 15 W/L at 20 °C) increased the antimicrobial activity of chlorine with an additional 1-log reduction in *Salmonella* Typhimurium.

Recently, Sagong *et al.* [128] studied the effect of ultrasound (40 kHz, 30 W/L for 5–60 min) combined with organic acids (malic acid, lactic acid, or citric acid, 0.3–2% for 5 min) at ambient temperature on *E. coli* O157:H7, *S.* Typhimurium, and *Listeria monocytogenes* in lettuce. The results revealed that the combined treatment resulted in additional 0.8 to 1.0 log reduction compared with individual treatments, without impairing colour or texture. Ultrasonic (25 kHz, 200–600 W for 1–30 min) inactivation efficacy of *Alicyclobacillus acidiphilus* and *Alicyclobacillus acidoterrestris* in apple juice was also investigated by Wang *et al.* [129]. It was concluded that *A. acidoterrestris*, seemed more sensitive (microbial reduction of 4.56 log cycles at 600 W for 30 min) to ultrasound treatments than *A. acidiphilus*. Yuan *et al.* [130] also studied the effect of ultrasound treatments (20–24 kHz, 60–900 W for 10–60 min) on *A. acidoterrestris* in apple juice. In general, inactivation of the cells was more pronounced at an elevated power level and as the processing time increased. Approximately 60% of the cells were inactivated after treating the apple juice with 300-W ultrasound for 30 min. The reduction reached more than 80% when the juice was processed for 60 min.

Nevertheless, Cheng *et al.* [131] showed that ultrasound (35 kHz for 30 min) alone or combined with carbonation was not effective in the reduction of yeast and mould in guava juice.

E. coli and *S. cerevisiae* were reduced by >99% after ultrasonication (20 kHz and 750 W) in a salt solution and milk, while *Lactobacillus acidophilus* was reduced by 72% and 84% for each suspension media, respectively [125]. Another study published by Bermúdez-Aguirre and Barbosa-Cánovas [53] proved TS (24 kHz, 400 W at 63 °C) to be a viable technology capable of destroying *Listeria innocua* ATCC 51742 (4.9 log reduction) in fat free milk. Noci *et al.* [132] also used TS (24 kHz at 55 °C) to inactivate *L. innocua* in milk samples. A treatment time of 2.7 min allowed approximately 1-log cycle reduction. Stanley *et al.* [133] also reported that increases in sonication treatment time, intensity (9.5, 21.8 and 49.2 W/cm^2), and temperature (ice water bath and 40 °C water bath) led to increased lethality of *E. coli* O157:H7 suspended in salt solutions. A reduction in *Staphylococcus aureus* was observed when orange juice was exposed to TS (30 kHz at 55 °C) for 5, 10, and 20 min, achieving 0.8-, 1.8-, and 3.3-log cycle reductions, respectively [134]. Ugarte-Romero *et al.* [135] performed inactivation experiments with *E. coli* K12 and temperature ranging from 40 °C to 60 °C and the results showed that sonication increased cell destruction by 5.3-log, 5.0-log and 0.1-log cycles at 40, 50 and 60 °C, respectively. In another study, 3-log cycle inactivation of *Zygosaccharomyces bailii* in orange juice by TS (20 kHz at 55 °C) was observed by Earnshaw *et al.* [24].

Ordóñez *et al.* [56] used TS (20 kHz, 160 W at 5 to 62 °C) for the inactivation of *Streptoccocus faecium* and *Streptoccocus durans*. The combination of ultrasound and heat was significantly more effective in inactivating these bacteria than each method used alone. Ciccolini *et al.* [136] reported in a study (20 kHz at 45 to 55 °C) with *S. cerevisiae* suspended in water that ultrasonic waves were not able to destroy yeast cells, but at high temperature the synergistic effect between temperature and ultrasound was observed. Kuldiloke and Eshtiaghi [137] reported in a study with *S. cerevisiae* that ultrasound (20 kHz at ≥80 W) combined with moderate temperature (50 °C for 15 min) resulted in up to 6-log inactivation of the yeast in orange juice. Recently, Adekunte *et al.* [138] treated tomato juice with ultrasound (20 kHz, 0.33–0.81 W/mL at 30.6–39.9 °C) and obtained up to 5-log reductions reduced cells in *Pichia fermentans*.

Haughton *et al.* [139] evaluated the potential of high and low ultrasound intensities (24 kHz, 20000 W/L at 53 °C; 40 kHz, 20 W/L at 53 °C) for improving the microbial safety of poultry. No viable

Campylobacter or enterobacteriaceae were detected, and total viable counts (TVC) were reduced by 2.49 log cfu/g by TS with high intensity. The low intensity TS reduced enterobacteriaceae and TVC populations by 2.74 and 1.69 log cfu/g, respectively. Alexandre *et al.* [140] studied the impact of TS as an alternative to blanching watercress and strawberry. The results showed that TS (35 kHz, 120 W for 3 min) at 65 °C was more effective than heat blanching on the reduction of total coliforms in watercress. TS at 60 °C also reduced total mesophilic bacteria in strawberries. Cao *et al.* [141] also reported 0.88-log reductions of aerobic microorganisms in strawberries with the application of ultrasound (25 kHz for 10 min). Recently, Muñoz *et al.* [142] showed that TS (24 kHz, 400 W for 2.9 min at 40 °C or 5 min at 50 °C) and pulsed light (360 µs, 3 Hz and 4.03 J/cm^2 or 5.1 J/cm^2) hurdles allowed almost 6-log reductions of *E. coli* in apple juice.

In another study, combined sonication (20 kHz at 450–2000 W) with a pressure of 200 kPa (i.e. using manosonication) at 40 °C reduced the D-value of *L. monocytogenes* from 4.3 to 1.5 min [54]. Lee *et al.* [143] showed that four different treatments with ultrasound (100–500 kPa, 20 kHz at 40–61 °C) significantly shortened treatment times to achieve *E. coli* 5-log reductions. Moreover, scanning electronic micrographs of sonicated samples presented extensive cell damage and breakage.

Combining ultrasound with direct steam injection resulted in a higher inactivation of *Bacillus stearothermophilus* spores [144]. Raso *et al.* [61] reported that MS (500 kPa, 20 kHz for 12 min) reduced about 99% of *Bacillus subtilis*. In another type of application, ultrasound (21.2 kHz) enhanced a sanitizer efficacy in the reduction of *E. coli* O157: H7 on spinach leaves by 0.7 to 1.1 log cycles compared to the treatment using the sanitizer only [145].

Arroyo *et al.* [146] characterized the resistance of *Cronobacter sakazakii* to MS (0–300 kPa, 20 kHz at 450 W) and concluded that *C. sakazakii* cells treated by MS present sublethally injured outer membranes.

Sert *et al.* [147] studied the effects of ultrasonic treatment and storage temperature on egg quality. The lowest yolk and albumen total mesophilic bacteria values (2.403 log cfu/g; control- 2.565 log cfu/g) were observed in eggs treated with ultrasound (35 kHz, 140 W for 30 min) at 30 °C.

Most of these works report a comparison between the effect of a traditional processing treatment and a treatment additionally using ultrasound on enzymes and microorganisms. Efforts to reduce

the treatment temperature are made in some studies. Apart from a strong improvement provided by ultrasound in the reduction of several enzymes and microorganisms in combined treatments, further investigation is still necessary, since there is a lack of process combination and optimization studies. Thus, understanding the behaviour of each enzyme and microorganism, in different food matrices, contributes to the complete implementation of this technology at an industrial level, ensuring food preservation and at the same time meeting the demand for high quality foods. Nevertheless, the implementation costs of this type of technology must also be taken into account, since it may compromise its future applications.

13.5 Ultrasound Effects on Food Quality Attributes

There are several types of ultrasound food applications that may result in food products of higher quality. Vercet *et al.* [64] reported that applying heat and ultrasound under moderate pressure (20 kHz, 196 kPa, at 40 °C for 12 s) on milk allowed the production of yoghurts with rheological properties superior to those produced with untreated milk, since ultrasound caused higher level of protein denaturation.

Ertugay *et al.* [148] studied the effect of ultrasound treatment (20 kHz at 55 °C) on milk homogenization and particle size distribution of fat. It was found that ultrasound treatment is an effective system for the reduction of fat globule size compared with the conventional homogenization. In another study, Bojilskov *et al.* [149] also obtained a successful milk homogenization after applying ultrasound (30 kHz at 20 °C) with different probe diameters (7 mm and 10 mm). Bermúdez-Aguirre *et al.* [150] also concluded that TS (24 kHz at 400 W) can pasteurize and improve some sensorial milk characteristics such as colour and appearance without the use of intensive heat treatments. Moreover, Cruz *et al.* [151] revealed that TS (20 kHz at 125 W) was a better blanching process compared with heat-only treatment, since watercress vitamin C content was maintained at higher levels. The same authors also concluded that TS treatment improved the colour of the blanched watercress [152]. Rawson *et al.* [153] also reported higher values of ascorbic acid (98.6%) and lycopene (106.68%) in watermelon juice treated with TS at low temperature conditions (20 kHz at 25–45 °C).

Lee *et al.* [154] evaluated the effect of MTS (400 kPa, 70 °C at 30 s) on the quality of orange juice during storage at 4 °C. They reported that in the manothermosonicated juice, vitamin C exhibited a slower degradation rate when compared with the thermal pasteurized one. In a recent study, Alexandre *et al.* [140] reported an equal or better firmness of thermosonicated (35 kHz, 120 W, at 65 °C for 3 min) strawberries compared with the heat blanched samples. Also in the same study, strawberries sonicated at 15 °C retained the total content of anthocyanins. In another study, reporting the effects of ultrasound on meat products, the ultrasound, after prolonged exposure, produced the release of myofibrillar proteins improving meat water binding capacity, tenderness and cohesiveness [155]. Nevertheless, Cheng *et al.* [131] found that guava juice treated by ultrasound (35 kHz for 30 min), significantly changed the total colour difference (TCD) values, alone (TCD=0.84) or in combination with carbonation (TCD=1.62), due to a decrease in lightness and an increase in a and b values.

In another study, Sales and Resurreccion [156] quantified the synergistic enhancement of phenolics and antioxidants in peanuts by combinations of ultrasound (40–20 mW/cm^3 for 4–12 min) plus ultraviolet radiation (UV) treatments, compared with separate ultrasound or UV. The results revealed that bioactive phenolics, *trans*-resveratrol, *trans*-piceid, and *p*-coumaric-, caffeic-, and ferulic-acids, achieved maximum increases with combined ultrasound-UV, compared with ultrasound or UV alone. On one hand, the UV induces an increase in enzymes responsible for the biosynthesis of secondary metabolites such as flavonoids, which act as UV screens preventing UV-induced damage in the genetic material of plant cells. On the other hand, the ultrasound has the capacity to release enzymes from cells for the secondary metabolite biosynthesis due to mechanical stresses and microstreaming induced by acoustic cavitations. Moreover, Caminiti *et al.* [157] studied the impact of selected combinations of non-thermal processing technologies on the quality of an apple and cranberry juice blend, and concluded that the combinations, in which MTS (20 kHz, 750 W, 400 kPa, at 58 °C for 8.4 min) was included, adversely affected the odour and flavour of the juice, probably due to the production of free radicals. Other drawbacks of ultrasound include the loss of texture [158], rupture of skin in berries at high doses [159] and loss of phytonutrients [160] in some food products.

Thus on one hand, and depending on the type of processing treatment and food product, the ultrasonic treatment can improve product quality. On the other hand, some desirable attributes can be negatively affected. Thus, a compromise between advantages and drawbacks must be found that allows quality optimization.

13.6 Conclusions

Ultrasound treatments can be combined with and/or replace traditional thermal processes with an objective of producing safer food products with higher nutritional and sensory quality. Besides allowing a successful reduction of treatment temperature and showing a high versatility, being adaptable to different processing operations, a lack of standardization in ultrasound equipment configuration, operating frequencies and power levels makes meaningful comparisons between different studies difficult to achieve. Further investigations in different food products and treatment combinations with duly controlled process conditions are required in order to obtain a better understanding of the benefits and drawbacks of ultrasound treatments. Such information will allow economically feasible production-scale implementation of ultrasound technologies in the food industry.

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