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Engineering Foods for Bioactives Stability and Delivery

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Engineering Foods for Bioactives Stability and Delivery

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Preface

Dietary trends, nutritional recommendations and more stringent labeling regulations are placing increasing demands on the food industry. On the other hand, an understanding of food materials science and its impact on food processing and product development, and extending the understanding to nutrient delivery and gastric engineering for optimal performance in the gastrointestinal tract, provides significant new opportunities and challenges for food manufacturers. The interest in a broader food and health context at the interface of food science, food engineering, nutritional sciences, biological sciences, medicine and science in general has coincided with much research for mining and identification of numerous hydrophilic and lipophilic bioactive compounds. Food materials science has also been developed to a stage where food materials can be designed and produced to protect sensitive components, preserving their stability and controlled delivery in complex food products. Such rationally designed delivery systems must meet high safety and efficacy requirements and regulations, as well as economic viability criteria and consumer acceptance.

The present book introduces technologies and ways to engineer delivery systems for the stabilization of bioactive components in food ingredients and foods. The book also highlights and reminds readers about the importance of understanding physicochemical aspects of food stability at low water or low temperature conditions. Bioactive or nutraceutical components play a major role in the impact of food on health, but to ensure their optimal performance, food engineers and those involved in the development of processes and products must carefully consider extraction from raw materials, incorporation in foods, protection, stability during processing, shelf life and digestion, as well as release bioaccessibility and bioavailability in delivery. The present book discusses various topics, including *Recovery Technologies for Lipophilic Bioactives* (by C. Saldanha do Carmo, A.T. Serra, C.M.M. Duarte); *Recovery Technologies for Water-soluble Bioactives: Advances in Membrane-based Processes* (by Alfredo Cassano); *Industrial Production of Active Probiotics for Food Enrichment* (by Sai Swaroop Dalli, Bijaya K. Uprety and Sudip K. Rakshit); *Microencapsulation Technologies* (by M.A. Augustin and L. Sanguansri); *Nanoencapsulation Technologies*

(by Yoav D. Livney); *Encapsulation Efficiency and Capacity of Bioactive Delivery Systems* (by Sinéad B. Bleiel, Robert K. Kent and André Brodkorb); *β-Lactoglobulin-Based Nano and Microparticulate Systems for the Protection and Delivery of Bioactives* (by Fatoumata Diarrassouba, Ghislain Garrait, Gabriel Remondetto and Muriel Subirade); *Crystallization* (by Nicolas Verhoeven, Tze Loon Neoh, Takeshi Furuta, and Hidefumi Yoshii); *Freezing and Freeze-drying* (by Yrjö H. Roos); *Spray Drying of Bioactives* (by Zhongxiang Fang and Bhesh Bhandari); *Protective Performance of Delivery Systems in Production, Shelf Life and Digestion* (by Stephan Drusch and R. Wilde); *Food Extrusion* (by Zeki Berk), *Non-thermal Stabilization Processes* (by Gustavo Barbosa-Cánovas); *Chemical Stability: Browning and Oxidation* (by Naritchaya Potes and Yrjö H. Roos); and *Improvement of Bioaccessibility and Bioavailability: From Molecular Interactions to Delivery Systems* (by Maarit J. Rein and Marcia da Silva Pinto). These chapters cover general properties of food systems and technologies available for long-term stabilization of food ingredients and foods as delivery systems.

Our book aims to provide students, researchers and food professionals in general with information and insights essential for the successful design, production and utilization of food as a vehicle for delivering health-promoting components, without compromising the pleasure of the culinary experience. The contents extend to bioaccessibility and bioavailability of compounds, which represents an expanding future area of gastronomic and nutritional engineering of foods.

Cork, Ireland — Yrjö H. Roos
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Chapter 1
Recovery Technologies for Lipophilic Bioactives

C. Saldanha do Carmo, A.T. Serra and C.M.M. Duarte

Abstract The market for natural bioactive ingredients is one of the most attractive at the moment, and natural lipophilic compounds of various structures and functionalities provide excellent molecules for the production of nutraceuticals, functional foods, and food additives. The use of these products in various commercial sectors and the consumer demand for "natural", together with increasingly strict environmental regulation related to the use of organic solvents, particularly with products for human consumption, have created a great challenge for the natural ingredients industry. In order to address the need for safer and healthier foods and to offer competitive products aimed at meeting consumer expectations, many scientists and manufacturers are making genuine efforts to explore new "green sustainable processes" for obtaining bioactive ingredients. In this chapter, both conventional and new alternative techniques for the isolation of high-value lipophilic bioactives from different natural matrixes are presented. One new technology, supercritical fluid extraction (SCFE), is reviewed in greater detail. The practical issues associated with each extraction method are also discussed, as well as the potential for upscaling of the technology.

Keywords Lipophilic bioactive compounds · Conventional and Non-conventional extraction · Supercritical CO_2 extraction

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1.1 Introduction

Nowadays, there is increasing evidence concerning the physiological, nutritional, and medicinal benefits to human health from various natural products, as well as the possible harmful effects of the use of certain synthetic products. The market for bioactive natural ingredients is among the most attractive at the moment, and natural lipophilic compounds of various structures and functionalities provide excellent molecules for the production of active ingredients for functional foods and food additives (e.g., preservatives, flavours, or colourants).

Extracts from natural sources play an important role, and the different parts of plants such as seeds, leaves, flowers, berries, bark, and roots represent the major sources of natural extracts, although some extracts may be obtained from animal sources.

In order to address the need for safer and healthier products and to compete in meeting consumer expectations, manufacturers must adopt the most suitable procedure for producing the natural ingredients. Conventional extraction involves the use of solid–liquid techniques that typically rely on organic solvents, which present several drawbacks, including toxic residues, chemical transformation of extracts, and toxic wastes. This has led to the development of more innovative approaches for the production of the desired compounds. The selection of an extraction method is dependent on the type of compound to be extracted, and it should take into account the potential for scaling up of the technology.

The aim of this chapter is to summarize and provide an overview of the main lipophilic bioactives and their characteristics, sources, and methods of extraction from natural matrices. As supercritical carbon dioxide extraction has been shown to be an effective "green" alternative for organic solvent-based extraction of non-polar compounds, supercritical fluid technology will be discussed in greater detail.

1.2 Lipophilic Bioactives

Lipophilic bioactives constitute a wide range of substances with a variety of functions, including flavour, antimicrobial, antioxidant, and several health-promoting properties. The group of lipophilic bioactives is very diverse in terms of molecular properties (e.g., molecular weights, structures, functional groups, polarities, and charge), leading to different physicochemical and physiological properties including solubility, physical state, rheology, optical properties, chemical stability, surface activity, and bioactivity (McClements et al. 2007).

The most important classes of lipophilic bioactives are briefly presented below, including their physicochemical characteristics, bioactivity, and main sources. The chemical structures of various bioactives are schematically presented in Table 1.1.

Table 1.1 Chemical structure of some bioactives [PubChem database (Kim et al. 2015)]

Bioactives	Structures
Carotenoids	β-carotene Lycopene Lutein Zeaxanthin
PUFAs	DHA EPA
Tocopherols	α-tocopherol β-tocopherol γ-tocopherol δ-tocopherol
Squalene	Squalene
Essential oils	Thymol Carvacrol Linalool

1.2.1 Carotenoids

Carotenoids, the most significant and extensive group of pigments found in nature (Brunner 2005a), are a large group of lipophilic compounds that give a range of colours in foods from yellow to red. Furthermore, it has been strongly suggested that consuming carotenoid-rich foods reduces the risk of several types of disease, including cancer (Ziegler 1989; Gerster 1993; Astorg 1997), cardiovascular disease (Bendich 1994; Kohlmeier and Hastings 1995; Mayne 1996; Sesso et al. 2005;

Riccioni 2009), age-related macular degeneration (Seddon et al. 1994), cataracts (Jacques and Chylack 1991; Snodderly 1995; Mayne 1996; Olmedilla et al. 2003), diseases related to low immune function (Bendich 1994; Chew 1995; Meydani et al. 1995), and other degenerative diseases (Stahl and Sies 2005; Perera and Yen 2007).

Carotenoids are polyenes consisting of 3–13 conjugated double bonds and sometimes six carbon ring structures at one or both ends of the molecule (Cavalcanti et al. 2013). They can exist in *cis* and *trans* forms, but *cis* isomers are less stable than the *trans* forms due to stoichiometric conformation. Thus, the majority of natural carotenoids are in the all-*trans* configuration (McClements et al. 2007). Based on their structure, carotenoids are divided in two classes: (i) carotenes, which are pure polyene hydrocarbons containing only carbon and hydrogen atoms, and include acyclic lycopene and bicyclic β- and α-carotene; and (ii) xanthophylls, containing oxygen in the form of hydroxyl (lutein), epoxy (violaxanthin), and oxo (canthaxanthin) groups (Sajilata et al. 2008). The most abundant pigments in nature are β-carotene, lycopene, lutein, and zeaxanthin (de Paz et al. 2012).

Carotenoids are found in a large variety of natural sources: vegetables, animals, bacteria, yeast, and microalgae. About 90 % of the carotenoids in the human diet and body are β- and α-carotenes, which are commonly found in yellow-orange vegetables and fruits; α-cryptoxanthin is present in orange fruits, lutein is provided by dark green vegetables, and lycopene is obtained from tomatoes and their by-products (Rao and Rao 2007).

The carotenoids are the main dietary source of vitamin A precursors and antioxidants (de Paz et al. 2012). Although β-carotene is the main compound with pro-vitamin A activity, any carotenoid with at least one unsubstituted β ring, such as α-carotene or β-cryptoxanthin, has the added advantage of being able to be converted to vitamin A. Even though lycopene is a carotenoid with no pro-vitamin A activity, it is an important antioxidant and free radical scavenger (Pingret et al. 2013). Processed foods are frequently fortified with carotenoids such as lycopene to increase their nutritive value and/or enhance their attractiveness (Wang and Weller 2006). The major sources of lycopene are ripe tomatoes, and tomato products and by-products (skins and seeds) (Burton-Freeman and Reimers 2011).

1.2.2 Polyunsaturated Fatty Acids (PUFAs)

The polyunsaturated fatty acids (PUFAs) contain more than one double bond in their structure. PUFAs can be classified into various groups by their chemical structure: methylene-interrupted polyenes, conjugated fatty acids, and other polyunsaturated fats. The methylene-interrupted polyenes comprise the ω-3 essential fatty acids (hexadecatrienoic acid, α-linolenic acid, stearidonic acid, etc.), ω-6 fatty acids (linoleic acid, γ-linolenic acid, eicosadienoic acid, etc.), and ω-9 fatty acids (oleic acid, erucic acid, mead acid, etc.). The conjugated fatty acids have two or more conjugated double bonds, for example, linoleic acids (rumenic

acid) and linolenic acids (β-calendic acid). Pinolenic acid and podocarpic acid are examples of other PUFAs (McClements et al. 2007).

There is an extensive body of scientific literature supporting the positive effects of ω-3 fatty acids on human health. Important natural sources of ω-3 PUFA are marine organisms (fish, seafood, algae, and other marine sources) directly or indirectly fed from marine phytoplankton, the primary producer of ω-3 in the trophic chain (Rubio-Rodriguez et al. 2010), and selected seed plants and other marine sources (Sahena et al. 2009b). Consumption of ω-3 fatty acids can reduce the risk of heart disease (Hooper et al. 2006) and high blood pressure (Lungershausen et al. 1994), prevent blood clots, protect against cancer, and alleviate depression (Von Schacky et al. 1999). With regard to ω-6 fatty acids, they are often used to develop pharmaceutical drugs and for the treatment of atherosclerosis, asthma (Hodge et al. 1998; Oddy et al. 2004), arthritis (Geusens et al. 1994), vascular disease, thrombosis (Yamashita et al. 2005), immune-mediated inflammatory disorders (Fritsche 2006), and cancer (Cunnane 2003; Simopoulos 2002, 2008). Unlike ω-3, the ω-6 and ω-9 fatty acids are not classified as essential fatty acids, as they can be synthesized by the human body from unsaturated fatty acids. Among all compounds, particular attention has been focused on concentrates of EPA (*all-cis*-5,8,11,14,17-eicosapentaenoic acid) and DHA (*all-cis*-4,7,10,13,16,19-docosahexaenoic acid) due to their pharmaceutical value (Cavalcanti et al. 2013).

1.2.3 Tocopherols and Tocotrienols

Tocopherols constitute a class of chemical compounds that includes various methylated phenols. Tocopherols are extremely valuable compounds because of their vitamin bioactivity and antioxidant capacity (Lee et al. 2000). They are abundant in seeds and leaves of plants. γ-Tocopherol is present at high concentrations in seed oils (olive, sunflower, corn, and soybean) and α-tocopherol in leaf lettuce (Cavalcanti et al. 2013).

Vitamin E includes both tocopherols and tocotrienols that occur in groups of four (α, β, γ, δ) lipophilic antioxidants synthesized by photosynthetic organisms. The isomer with the highest vitamin E activity is α-tocopherol, and it has become an important additive in numerous food products. However, although it has a number of biological properties, it can cause indigestion, thus affecting its bioavailability in the intestine. Many biological functions related to tocopherol consumption have been identified, including relief of stress (Liu 2005) and premenstrual symptoms (London et al. 1983), prevention of cellular damage (Marubayashi et al. 1986), improved blood circulation (Kunisaki et al. 1998), tissue regeneration (Andıran et al. 2000), and intermittent claudication (Haeger 1974). Additionally, the antioxidant activity of tocopherols is associated with inhibition of membrane lipid peroxidation and the elimination of reactive oxygen species (Folmer et al. 2009).

1.2.4 Phytosterols

Phytosterols and phytostanols, the saturated form of phytosterols, are steroidal compounds similar to cholesterol but of plant origin. They vary only in their carbon side chains and/or presence or absence of a double bond (Ostlund 2002). Vegetable oils and vegetable oil-containing products are rich sources of phytosterols (Cavalcanti et al. 2013). Large-scale isolation of phytosterols is conducted mainly in two major raw materials, vegetable oils and tall oil (Fernandes and Cabral 2007).

The most common phytosterols in the human diet are β-sitosterol (65 %), campesterol (30 %), and stigmasterol (3 %). Among phytostanols, the most common in the human diet are sitostanol and campestanol, which together constitute about 5 % of dietary phytosterols (Cavalcanti et al. 2013). They are known to reduce low-density lipoprotein (LDL) serum cholesterol levels (Jones et al. 1997), and thus foodstuffs containing phytosterols are widely used as a dietary therapeutic option for reducing plasma cholesterol and the risk of atherosclerosis (Moghadasian and Frohlich 1999). Grain products, vegetables, fruits, and berries are not as rich in phytosterols as vegetable oils, but they can also be significant sources due to their high consumption, reaching 150–450 mg/day (Cavalcanti et al. 2013).

Phytosterols play a major role in several areas, including pharmaceuticals (production of therapeutic steroids), nutrition (anti-cholesterol additives in functional foods, anti-cancer properties), and cosmetics (due to their anti-inflammatory activity, which is an interesting property for anti-ageing products).

1.2.5 Squalene

Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) is a biosynthetic precursor to all steroids (He et al. 2001). Squalene is a symmetrical 30-carbon polyprenyl compound containing six prenyl units. Shark liver oil (*Squalus* spp.) is considered the richest source of squalene, and it is an intermediate metabolite in the synthesis of cholesterol. In humans, about 60 % of dietary squalene is absorbed and distributed in all tissues, being one of the major components in human epidermis lipids. Its major function is acting as an antioxidant, protecting the skin from lipid peroxidation (Briganti and Picardo 2003).

It is hypothesized that the lower risk of various cancers associated with high olive oil consumption could be due to the presence of squalene (Smith 2000). Research has also suggested that squalene has chemopreventive effects against colon cancer (Rao et al. 1998), and it has been recognized as having cholesterol-lowering effects (Smith et al. 1998; Kelly 1999) (Khor and Chieng 1997).

The traditional source of squalene is primarily shark (*Centrophorus squamosus*) and whale (*Physeter macrocephalus*) liver oils (Jahaniaval et al. 2000).

1.2.6 Essential Oils

Essential oils contain an important group of bioactive organic compounds that are responsible for the aroma and are involved in the defence mechanisms of many plants. EOs contain 85–99 % volatile and 1–15 % non-volatile components. The volatile constituents are a mixture of terpenes, terpenoids, and other aromatic and aliphatic constituents of low molecular weight. Terpenes are made from combinations of several five-carbon-base (C5) units called isoprenes. The main terpenes are the monoterpenes (C10) and sesquiterpenes (C15). Terpenoids are terpenes containing oxygen. Monoterpenes, formed from the coupling of two isoprene units, are the most representative molecules, constituting 90 % of the EOs. Aromatic compounds are derived from phenylpropane. Aldehydes (cinnamaldehyde), alcohols (cinnamic alcohol), phenols (eugenol), methoxy derivatives (anethole, estragole), and methylenedioxy compounds (myristicin, apiole) are examples of their aromatic components (Sánchez-González et al. 2011).

These compounds can be stored in several organs, such as flowers (orange, bergamot), leaves (lemongrass, eucalyptus, and menthe), bark (cinnamon), wood (sandalwood, rosewood), rhizomes (curcuma, ginger), fruits (star anise, fennel), and seeds (nutmeg) (Pereira and Meireles 2010).

Essential oils generally have a broad spectrum of bioactivity owing to the presence of several active ingredients that work through various modes of action. Known for their antiseptic—i.e., bactericidal (Friedman et al. 2002), virucidal (Garcia et al. 2003), and fungicidal (Ranasinghe et al. 2002)—and medicinal properties and their fragrance, they are used in embalmment, for food preservation, as antimicrobial (Burt 2004) and analgesic remedies, sedatives, anti-inflammatory (Silva et al. 2003) and spasmolytic (Sadraei et al. 2001) agents, and as a local anaesthetic (Bakkali et al. 2008). These properties have made essential oils an attractive candidate for use as ingredients in cosmetics, food, and pharmaceutical products (Pereira and Meireles 2010).

1.3 Methodologies for Extraction of Lipophilic Bioactives

Various extraction procedures have been used for the recovery of bioactive compounds from natural matrices. These include both conventional and non-conventional methods.

1.3.1 Conventional Extraction Techniques

Soxhlet extraction, hydro-distillation, and maceration with an alcohol–water mixture or hot fat are conventional techniques applied for the extraction of nutraceuticals from natural matrices using heat and/or agitation (Wang and Weller 2006; Azmir et al. 2013).

The Soxhlet extractor was first proposed in 1879 by Franz Ritter von Soxhlet, a German chemist, and it has since been widely adopted as a standard technique and the primary reference for assessing the performance of new extraction alternatives (Wang and Weller 2006; Azmir et al. 2013). With the exception of the extraction of thermolabile compounds, Soxhlet extraction is a generally well-established method, with performance exceeding that of other conventional extraction techniques. Briefly, a small amount of dry sample is placed in a thimble filter, which is then placed in a thimble holder above a distillation flask containing the solvent of interest. When the liquid reaches the overflow level, a siphon aspirates the solution passing through the thimble holder and unloads it back into the distillation flask, carrying extracted solutes into the bulk liquid. In the solvent flask, solute is separated from the solvent using distillation. The solute is left in the flask, and fresh solvent condensed by reflux after evaporation is passed back into the plant solid bed. The operation is repeated until complete extraction is achieved (Wang and Weller 2006; Azmir et al. 2013). The main criticism with this method is the length of time required to complete the process (Luque de Castro and Priego-Capote 2010).

Hydro-distillation is another traditional method for extraction of bioactive compounds and essential oils from plants. With this technique, organic solvents are not used, and it can be performed without drying the plant materials. There are three types of hydro-distillation: water distillation, water and steam distillation, and direct steam distillation (Palma et al. 2013). In water distillation, the solid matrix is sustained and packed in a still compartment and immersed in the boiling water or floating on it, depending on its density. In the water and steam distillation method, steam is also directly injected into the plant sample. Hot water and steam extract and release the bioactive compounds from the plant tissue. Indirect cooling by water condenses the vapour mixture of water and oil. The condensed mixture flows from the condenser to a separator, where oil and bioactive compounds automatically separate from the water. Lastly, in direct steam distillation, the solid matrix is supported on a perforated grid or screen inserted above the bottom of the still, but it is not in direct contact with the liquid water. The saturated steam flows up through the extracted solids, evaporating and gathering the volatile components (Palma et al. 2013), and the mixture is then condensed and separated as described above.

Hydro-distillation involves three main physicochemical processes: hydro-diffusion, hydrolysis, and decomposition by heat. This precludes its use for the extraction of thermolabile compounds, because some volatile compounds may be lost (Reverchon and Senatore 1992).

Maceration has become a popular and inexpensive way to extract essential oils and bioactive compounds, and it generally consists of several steps. First, the plant materials are ground into small particles to increase the surface area for proper mixing with the solvent. Next, an appropriate solvent, called menstruum, is added in a closed vessel. The liquid is then strained off, and the solid residue is pressed to recover as much occluded solution as possible. The strained and pressed liquids are

mixed and separated from impurities by filtration (Palma et al. 2013). In order to facilitate extraction, occasional shaking can be used, increasing the diffusion and the removal of concentrated solution from the sample surface, while carrying fresh menstruum inward for higher extraction yields (Azmir et al. 2013).

1.3.2 Non-conventional Extraction Techniques

The major drawbacks of conventional extraction procedures include long extraction time, low extraction selectivity, thermal decomposition of thermolabile compounds, and evaporation of large amounts of solvent (Azmir et al. 2013). To overcome these limitations, new and promising techniques have been explored.

1.3.2.1 Supercritical Fluid Extraction

The use of supercritical fluids (SCFs) is often highlighted as an important strategy within green chemistry to replace harmful organic solvents and to enable green sustainable technologies (Leitner and Poliakoff 2008). The critical point is characteristic of each substance and it is schematically illustrated in Fig. 1.1. The state of a substance is called supercritical when temperature and pressure exceed the critical values.

Going over the liquid-vapour line of a pure substance, the rise in temperature and pressure have different effects in each phase. When the temperature of a substance reaches its critical value, the densities of the gas and the liquid phases become identical, and the liquid is no longer distinguishable from the gas. At this point, only one phase exists and is now described as an SCF, whose properties range

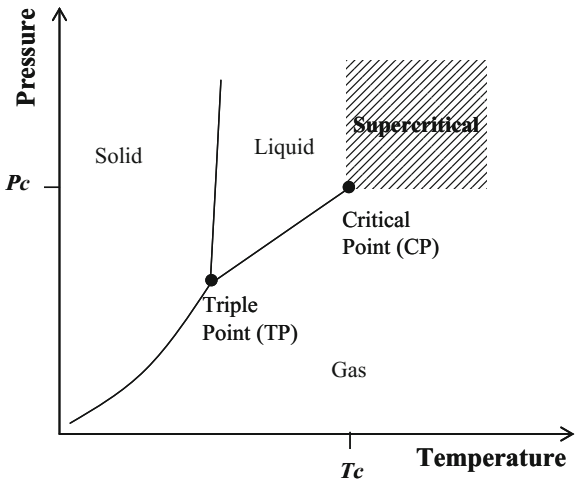

Fig. 1.1 Phase diagram for a pure component (Brunner 2005b)

Table 1.2 Orders of magnitude of main physical properties of SCFs [adapted from (Brunner 2005b)]

	Liquid	Supercritical	Gas
Density ($g.cm^3$)	1	0.1–0.5	10^{-3}
Diffusivity ($cm^2.s^{-1}$)	10^{-5}	10^{-3}	10^{-1}
Viscosity (Pa.s)	10^{-3}	10^{-4}-10^{-5}	10^{-5}

between those of gases and liquids, as shown in Table 1.2. Furthermore, SCFs are characterized by gas-like viscosities and solvating properties of a wide range of various organic solvents.

The most important feature of SCFs is that in the supercritical region (Fig. 1.1), minor variations in pressure and/or temperature lead to significant variation in density, which causes rapid changes in thermodynamic and transport properties.

Commensurate with the goal of environmentally benign processing is the use of liquefied or supercritical carbon dioxide (SC-CO_2) for non-polar to moderately polar solutes. Environmentally benign carbon dioxide in its supercritical state has demonstrated significant potential for the development of a wide range of alternative processes that totally or partially eliminate the need for some of the most commonly used organic solvents. In addition to this factor, the rapid mass transfer properties associated with the lower viscosity of SCF carbon dioxide can lead to more time-efficient production capabilities for various types of important industrial processes (King and Srinivas 2009).

The extraction of compounds from natural sources is the most widely studied application of SCFs. In fact, supercritical fluid extraction (SCFE) has significant advantages over conventional solvent methods: it is an environmentally friendly technique, it enhances extraction efficiency and selectivity, and avoids the expensive post-processing of the extracts for solvent elimination. For the above-mentioned -reasons, carbon dioxide (CO_2) is the most frequently used SCFE solvent. Extracts from supercritical processing with SC-CO_2 can be regarded as all natural, and the products allowed for food applications may have GRAS (generally recognized as safe) status. In addition to the advantages related to non-toxicity and mild operating conditions, the extraction system provides a light- and oxygen-free environment, thereby minimizing degradation and preserving their bioactivity and antioxidant properties.

Typical SCFE systems for solid and liquid extraction are presented in Figs. 1.2 and 1.3, respectively. Basically, the system consists of pumps for delivering solvent and modifiers (co-solvents) throughout the system and for raising the pressure of the recycled solvent, a high-pressure extractor, a pressure reduction valve, heat exchangers, compressors, and one or more separators in which the extract is collected and the solvent (e.g., CO_2) is depressurized and removed (Fig. 1.2) (Shi et al. 2012). A small amount of co-solvent (ethanol, water, etc.) increases the ability of SC-CO_2 to dissolve polar compounds (Reverchon and De Marco 2006).

Column fractionation can be done with a column operating in two different modes: cross-current and counter-current. The latter is the most popular. In such a

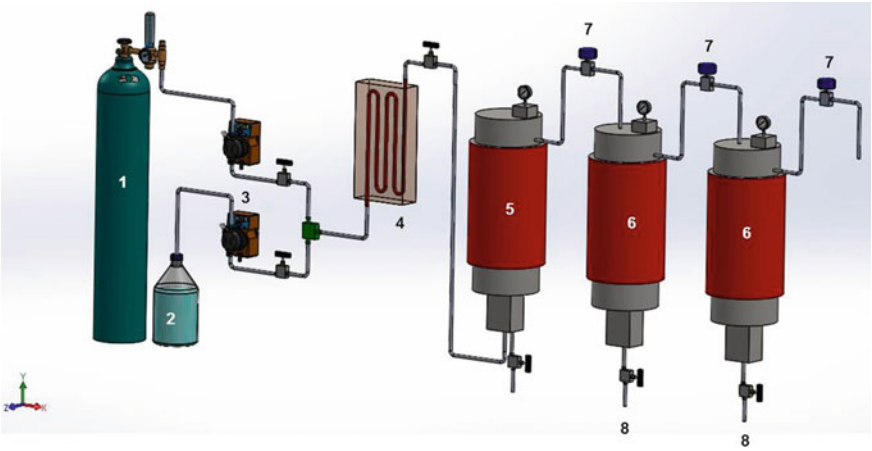

Fig. 1.2 Schematic diagram of a supercritical fluid extraction system (Solid-scFluid extraction) used to fractionate bioactive components. *1* CO_2 cylinder; *2* Co-solvent; *3* CO_2 and co-solvent pumps; *4* Heat exchanger; *5* Extraction vessel; *6* Separators; *7* Back pressure valves; *8* Extracts outlet

counter-current separation process, the components are distributed between the solvent (extract phase) and the liquid (raffinate phase) which flow in a counter-current manner through the column, reducing the amount of solvent needed and increasing throughput. Counter-current processing with SCFs extends the possibilities of separation processes like distillation, absorption and liquid–liquid extraction to the isolation and purification of components of low volatility, allowing separation of components with very similar properties (Fig. 1.3).

The industry has recently focused on "fractional separation", where the natural materials are extracted under relatively severe pressure and temperature conditions to remove all the desired components. The resulting fluid extract is then passed through a series of two, three, or four separator vessels in which the operating parameters (temperatures and pressures) in each vessel are set to selectively precipitate one specific component of interest. This can create a range of unique fractions with new application potential.

The past 20 years have seen an expansion of the "critical fluid" technology platform with respect to using or combining multiple types of unit operations and compressed fluids in both their sub- and supercritical states. For example, combining SCFE with fractionation methods using SCFs, and/or reaction chemistry in critical fluid media can produce a variety of extracts or products (King 2000).

Table 1.3 summarizes the advantages, disadvantages, parameters to control, and ease of scale-up of SCFE, in particular with carbon dioxide.

In summary, SC-CO_2 is suitable for applications in which (i) processing costs are not a limiting factor, (ii) conventional solvent extraction is restricted by environmental regulations, (iii) consumer demand or health considerations play an important role, (iv) a "natural" label on the product is required, and/or (v) the

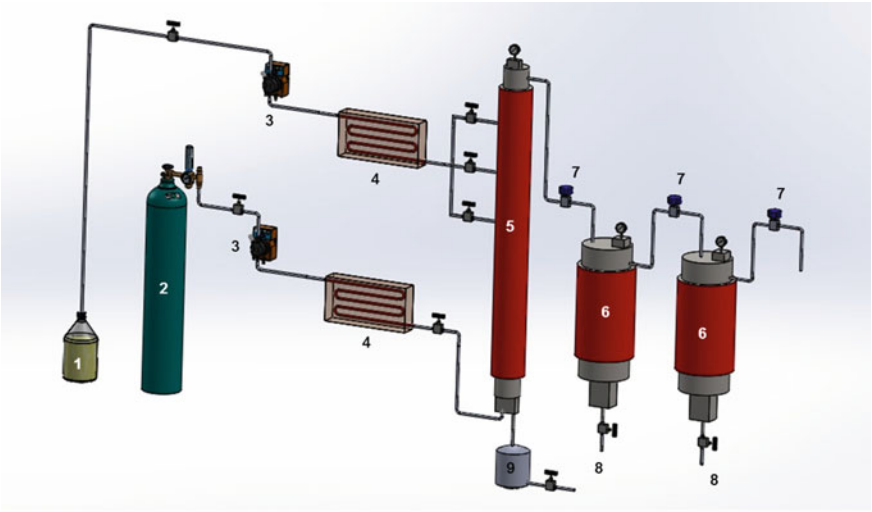

Fig. 1.3 Schematic diagram of a supercritical fluid extraction system (Liquid-scFluid extraction) used to fractionate bioactive components. *1* Feed; *2* CO_2 cylinder; *3* CO_2 and liquid pumps; *4* Heat exchangers; *5* Extraction column; *6* Separators; *7* Back pressure valves; *8* Extracts outlet; *9* Raffinate

application of traditional extraction techniques does not preserve the bioactive properties of the product. The major disadvantage of SCFE is the expensive equipment. With regard to the process itself, the drawbacks are the high pressure required and the associated hazards, which have hindered wider application.

SCFE has been commonly used for the extraction of flavours, essential oils, seed oils, antioxidants, and bioactives from several natural sources. Some of these applications have been reviewed extensively (Reverchon and De Marco 2006; Herrero et al. 2006a; Cheah et al. 2006; Díaz-Reinoso et al. 2006; Sahena et al. 2009a; Catchpole et al. 2009), and are summarized in Table 1.4.

1.3.2.2 Other Non-conventional Extraction Techniques

An overview of other non-conventional extraction techniques, including ultrasound-assisted extraction, microwave-assisted extraction (MAE), and pressurized liquid extraction (PLE), is presented in Table 1.5, along with the principles, main advantages, limitations, and information regarding the scaling up of each technology.

Table 1.3 Advantages, disadvantages, parameters to control and ease of scale-up of SCFE

Extraction methods	Advantages	Disadvantages	Parameters to control	Ease of scale-up
Supercritical fluid extraction (SCFE)	• Environmentally safe technology (Herrero et al. 2006b) • High selectivity, short times of extraction, lower pollution, and the use of non-toxic organic solvents (Wang and Weller 2006) • SFs have better transport properties than liquids because their viscosity depends on their density which, unlike liquid solvents, is adjustable by changing pressure and temperature (Sihvonen et al. 1999; Herrero et al. 2006b) • Sterilizing properties (Cheah et al. 2006)	• Low dielectric constant which suggests poor solvent power for polar materials (Wang and Weller 2006) • High pressure and the associated hazards, which have hindered a wider development (Herrero et al. 2006a) • Large number of variables to optimize (Cheah et al. 2006) • Strong dependence on matrix-analyte interactions (Cheah et al. 2006)	• *Matrix* particle size, porosity, location of the solute, moisture content, solubility • *Process* pressure and temperature (solubility), solvent flow rate (solvent-to-feed ratio)use of modifiers (co-solvents) (Mendiola et al. 2013)	Robust and economically viable process for industrial implementation (Fernandez-Ronco et al. 2013)
Supercritical carbon dioxide (SC-CO_2)	• Safe (Daintree et al. 2008) • Low cost (Daintree et al. 2008) • Ensure minimal alteration of the bioactive compounds and to preserve their curative or functional properties (Cavero et al. 2006) • Non-explosive (Wang et al. 2008; Wang and Weller 2006; Sahena et al. 2009b) • Non-toxic, inexpensive (Wang et al. 2008; Wang and Weller 2006; Sahena et al. 2009b) • Ability to solubilize lipophilic substances, and can be easily removed from the final products (Wang et al. 2008; Wang and Weller 2006; Sahena et al. 2009b) • Solvent-free extracts • This molecule is environmentally friendly and "generally recognized as safe" (GRAS) by FDA (U.S. Food and Drug Administration) and EFSA (European Food Safety Authority) • Allows the extraction of thermally labile or easily oxidized compounds (Herrero et al. 2010)	• Because of its low polarity, CO_2 is less effective in extracting highly polar compounds from their matrices (Herrero et al. 2006b) • The use of other solvent compounds is needed in order to enhance solubility and the selectivity of the process and they must be added only in small quantities (Sihvonen et al. 1999)		

Table 1.4 High-added-value compounds extracted with supercritical fluid extraction (Reverchon and De Marco 2006; Herrero et al. 2006a; Cheah et al. 2006; Díaz-Reinoso et al. 2006; Sahena et al. 2009a; Catchpole et al. 2009; Adil et al. 2007, 2008; Ibáñez et al. 2000; Nobre et al. 2009)

Bioactives	Raw materials
Essential oils	Bacuri fruit shells; Basil leaves; Black cumin; Carrot fruit; Chamomile flowers; Clove bud; Eucalyptus leaves; Ginger; Rose hip fruit; Juniper; Laurel leaves; Lemon balm; Lemon bergamot; Lemon eucalyptus; Lemon verbena; Lemongrass leaves; *Lippia alba;* Lovage leaves and roots; Marjoram leaves; Mint leaves; Oregano; Patchouli; Pennyroyal; Black pepper; Rosemary; Sage leaves; Savoury; Spiked thyme; Star anise; Sweet basil; Thyme; Tuberose concrete; Valerian root; Vetiver; Yarrow flowers
Seed oils	Black cumin seeds; Blackcurrant seed; Borage seeds; Caraway; Celery roots; Cinnamon; Coriander seeds; Daphne; Evening primrose seed; Fennel seeds; Grape seeds; Hemp seed; Rose hip seeds; Hyssop; Juniper; Kiwifruit seed; Lavender; Medlar seeds; Palm kernel oil; Peach seeds; Red pepper; Rose hip seed; Tea seed; *Vernonia* seeds
Carotenoids	Alfalfa leaf; Apricot pomace; Buriti fruit; Cardamom; Carrot; Crustaceans; Krill; Marjoram; Microalgae (Chlorella vulgaris, Nannochloropsis gaditana, Spirulina maxima, *Dunaliella salina*, *Haematococcus pluvialis*, *Phaffia rhodozyma*); Palm; Paprika; Pitanga; Rose hip; Sea buckthorn; Spirulina; Stinging nettle; Sweet potato; Tomato and tomato by-products (paste waste, skin); Watermelon
Tocopherols	*Aloe vera* leaves; Coriander seeds; Dill; *Espinheira santa*; Fresh bay; Grape seeds; Milk thistle; Olive oil industry by-products; Olive tree leaves; Palm; Paprika; Parsley; Pomegranate; Rice bran; Sacha inchi; Sesame seed, black; *Silybum marianum*; Soybean flakes; Soybean oil by-product; Spearmint; Tomato; Wheat germ
Squalene	*Espinheira santa*; Indian almond leaves; Olive oil industry by-products; Shark liver oil; Tropical almond leaves and seeds
Fatty acids	Brown seaweed; Cardamom seed; Corn bran; Cotton seed; *Cunninghamella echinulata*; Grape seeds; Ground beef; Mackerel; Northern shrimp; Oat Bran; Peanuts; Palm kernel; Pecan; Pistachio; Pupunha; *Pythium regulare*; Rapeseed; Rice bran; Rose hip seed; Safflower; Sardine oil; Saw Palmetto berries; Soybean; Sunflower; Wheat germ

1.4 Extraction of Lipophilic Bioactives

1.4.1 Carotenoids

Carotenoid extraction has traditionally been performed using organic solvents that are associated with the known problems of low selectivity and adverse environmental impact. For these reasons, the use of SCF has been explored for their extraction. Because carotenoids show very low or moderate solubility in SC-CO_2, depending on the molecular structure (molecular weight and polarity), suitable modifiers (co-solvents) have been employed to enhance the solubility of the target compounds. The most frequently used co-solvent is ethanol, since its presence (in traces) in final products does not compromise their use in food, nutraceutical, and pharmaceutical

Table 1.5 Conventional solvent extraction and Non-conventional extraction techniques: principle of the method, advantages, disadvantages, parameters to control, and scale-up

Extraction method	Principle	Advantages	Disadvantages	Parameters to control	Scale-up
Conventional solvent extraction (SE)	• Pre-treated raw material is exposed to different solvents (Starmans and Nijhuis 1996) • Compounds of interest and other agents are extracted (Starmans and Nijhuis 1996) • Samples are usually centrifuged and filtered to remove solid residue (Starmans and Nijhuis 1996)	• Low production cost (Szentmihalyi et al. 2002) • Ease of operation (Szentmihalyi et al. 2002)	• Toxic solvents for humans and dangerous for the environment (Li et al. 2006) • Laborious extraction conditions (Li et al. 2006) • The solvent must be removed from the final extract, especially if the product is to be used in food applications (Starmans and Nijhuis 1996) • It requires an evaporation/ concentration step for recovery, using large amounts of solvent and extended time to carry out (Szentmihalyi et al. 2002) • Possibility of occurrence of thermal degradation due to high temperatures of solvents and long extraction times (Szentmihalyi et al. 2002)	• *Matrix* Matrix composition, sample size and extraction time • *Process* temperature, type of solvent and its characteristics (polarity, volume and flow rate) (Azmir et al. 2013)	• Not found
Pressurized liquid extraction (PLE) or accelerated SE (ASE) (Priego-Capote and Delgado de la Torre 2013)	• Accelerated SE and pressurized SE using organic liquid solvents at high temperature (423 K-473 K) and pressure (10-15 MPa) (Dunford et al. 2010) • As the temperature increases, the dielectric constant of the solvent decreases, thus lowering the polarity of the solvent (Abboud and Notario, 1999)	• Rapid extraction rate of compounds (Dunford et al. 2010) • The temperature can be used to match the polarity of a solvent to that of the compounds of interest to be recovered (Dunford et al. 2010) • The high pressure helps the extraction cells to be filled faster and forces liquid into the solid matrix (Plaza et al. 2010a) • Allow a faster extraction in which less amount of solvents are used and higher yields are obtained in comparison with traditional SE (Plaza et al. 2010a) • Allows the achievement of food-grade extracts when using water or other GRAS (generally recognized as safe) solvents,	• Not suitable for thermolabile compounds, as high temperature can have harmful effects on their structure and functional activity (Ajila et al. 2010)	• *Matrix* composition, sample size and extraction time • *Process* temperature, pressure, type of solvent and its characteristics (polarity, volume and flow rate)	• Scale-up to large production units not effective (Pronyk and Mazza 2009) • Difficulties in producing a system able to operate on a continual basis (Pronyk and Mazza 2009) • Most scale-up has occurred with older pressurized fluid extraction technology of SCFE (Pronyk and Mazza 2009) • The younger technologies of PLE extraction are gaining popularity in the research community (Pronyk and Mazza 2009)

(continued)

Table 1.5 (continued)

Extraction method	Principle	Advantages	Disadvantages	Parameters to control	Scale-up
		such as ethanol (Plaza et al. 2010a)			
Microwave-Assisted Extraction (MAE)	• Extraction works by heating the moisture inside the cells and evaporating, producing a high pressure on the cell wall (Routray and Orsat 2012) • The pressure builds up inside the biomaterial, which modifies the physical properties of the biological tissues (cell wall and organelle disrupter), increasing the porosity of the biological matrix (Routray and Orsat 2012)	• Better penetration of solvent through the matrix and improved yield of the desired compounds (Routray and Orsat 2012) • Internal and external heating without a thermal gradient, in which bioactives can be extracted efficiently and safely using less energy and solvent volume (Routray and Orsat 2012) • Reduced solvent consumption (Zhang et al. 2011) • Shortened extraction times (Simsek et al. 2012; Xiao et al. 2008) • Higher yields (Simsek et al. 2012; Xiao et al. 2008) • Lower environmental pollution (Simsek et al. 2012; Xiao et al. 2008)	• Modification of the chemical structure of target compounds (modification of bioactivity) (Zhang et al. 2011) • Additional filtration or centrifugation is necessary to remove a solid residue (Zhang et al. 2011)	• *Matrix* Size and moisture • *Process* Choice of solvent composition, solvent-to-feed ratio, power applied, extraction temperature and extraction time (Destandau et al. 2013)	• Poor reproducibility, poor control over process quality, complexity in scaling up (Li et al. 2013)
Ultrasonic-Assisted Extraction (UAE)	• Sound waves with frequencies higher than 20 kHz are mechanical vibrations in a solid, liquid and gas (Wang and Weller 2006) • Sound waves must travel in a matter, involving expansion and compression cycles during travel in the medium (Wang and Weller 2006) • When the ultrasound intensity is sufficient, the expansion cycle can create cavities or microbubbles in the liquid (Leighton 2007; Soria and Villamiel 2010; Esclapez et al. 2011) • The implosion of cavitation bubbles can hit the surface of the solid matrix and disintegrate the cells, causing the release of the desired compounds	• Reduction in extraction time (Leighton 2007; Soria and Villamiel 2010; Esclapez et al. 2011) • Higher recovery of targeted compounds with lower solvent consumption and/or faster analysis and bioactivity properties (Leighton 2007; Soria and Villamiel 2010; Esclapez et al. 2011) • Reasonable extraction efficiency (Leighton 2007; Soria and Villamiel 2010; Esclapez et al. 2011). • Facilitates leaching of organic and inorganic compounds from	• UAE should be carefully used in the extraction of unstable compounds (Zhao et al. 2006) • The presence of a dispersed phase contributes to the ultrasound wave attenuation, and the active part of ultrasound inside the extractor is restricted to a zone located in the vicinity of the ultrasonic emitter (Wang and Weller 2006)	• *Matrix* Moisture content, particle size, solvent • *Process* Frequency, pressure, temperature and sonication time (Pingret et al. 2013)	• UAE is attracting growing interest, and several industrial-scale reactors have already been successfully applied in the food industry (Tao and Sun 2013)

(continued)

Table 1.5 (continued)

Extraction method	Principle	Advantages	Disadvantages	Parameters to control	Scale-up
	(Leighton 2007; Soria and Villamiel 2010; Esclapez et al. 2011)	plant matrix (Herrera and de Castro 2005) • Effective mixing (Chemat et al. 2008) • Faster energy transfer (Chemat et al. 2008) • Thermal gradients and extraction temperature are reduced (Chemat et al. 2008) • Selective extraction (Chemat et al. 2008) • Compact equipment size (Chemat et al. 2008) • Quick control of extraction process (Chemat et al. 2008) • Start-up is quick (Chemat et al. 2008) • Higher production (Chemat et al. 2008) • Process steps are eliminated (Chemat et al. 2008)			

Table 1.6 Extraction of lycopene and **β**-carotene from tomato and tomato by-products using SC-CO_2 extraction and UAE

Extracted compound(s)	Source/raw material	Extraction conditions[a]	Yield (mg/100 g)/ Recovery[b] (%)	Observations	Reference
Supercritical CO_2 extraction					
Lycopene β-Carotene	Tomato (pulp and skin)	CO_2 T = 353 K; P = 27.6 MPa	Lycopene 64.4 mg/100 g 87 % β-Carotene 34.9 mg/100 g 92 %	Extracted product contained 65 % of lycopene and 35 % of β-carotene	Cadoni et al. (1999)
Lycopene β-Carotene	Tomato paste waste	CO_2 + 5 % EtOH T = 328 K/338 K, P = 30 MPa	Lycopene 9.1 mg/100 g 54 % β-Carotene 0.53 mg/100 g 50 %	Total of 100 % recovery of supercritical extracted carotenes was not possible because of the degradation of the product at higher temperatures	Baysal et al. (2000)
Lycopene	Tomato skins	CO_2 T = 383 K; P = 40 MPa	100 %	The addition of acetone (500μL) speeded up the beginning of the extraction, leading to a relative recovery of 94 % in just 15 min	Ollanketo et al. (2001)
Lycopene	Tomato sauce by-product	CO_2 T = 359 K; P = 34.47 MPa	0.7 mg/100 g 61 %	β-Carotene, α-carotene, α-tocopherol, γ-tocopherol and δ-tocopherol were also detected in extracts	Rozzi et al. (2002)
Lycopene β-Carotene	Tomato skins	CO_2 T = 350 K; P = 30 MPa	Lycopene 31 mg/100 g 80 % β-Carotene 6.9 mg/100 g 88 %	The yield of β-carotene extracted from the raw tomato waste material was 88 %	Sabio et al. (2003)

(continued)

Table 1.6 (continued)

Extracted compound(s)	Source/raw material	Extraction conditions[a]	Yield (mg/100 g)/ Recovery[b] (%)	Observations	Reference
Lycopene	Tomato (sun dried)	CO_2 + 10 % hazelnut oil T = 339 K; P = 45 MPa	60 %	Besides the advantages of having an improvement in the yields of the lycopene extract the presence of the vegetable oil as co-solvent contribute to avoid the degradation of the lycopene	Vasapollo et al. (2004)
Lycopene	Industrial tomato by-products	CO_2 T = 353 K; P = 46 MPa	31.4 mg/100 g	Other compounds such as tocopherols and β-carotene were detected in the extracts. The content of lycopene in the final product was 90 %	Vagi et al. (2007)
Lycopene	Tomato pomace	CO_2 + 16 % EtOH T = 330 K; P = 40 MPa	28.64 mg/100 g 93 %	The optimal conditions for antioxidant capacity were 330 K and 53 MPa. IC_{50} was used as index: 0.081 mg/mL; Antioxidant activity was evaluated	Huang et al. (2008)
Lycopene	Dried tomatoes + roasted hazelnut powder	CO_2 T = 333 K; P = 40 MPa	423 mg/100 g 72.5 %	Yield of oil extracted = 80 %	Ciurlia et al. (2009)
Lycopene	Tomato skins	CO_2 + 10 % EtOH + 10 % olive oil T = 348 K; P = 35 MPa	73.3 %	The addition of olive oil increases the solubility of lycopene	Shi et al. (2009)
Lycopene	Tomato industrial waste	CO_2 T = 333 K; P = 30 MPa	93 %	The recovery of trans-lycopene was faster and higher for the sample with lower content	Nobre et al. (2009)

(continued)

Table 1.6 (continued)

Extracted compound(s)	Source/raw material	Extraction conditions[a]	Yield (mg/100 g)/ Recovery[b] (%)	Observations	Reference
Lycopene	Tomato peel by-product + tomato seed	CO_2 T = 363 K; P = 40 MPa	56 %	44 % of lycopene remained in both the pipeline and the solid matrix. The presence of fatty acid content in the tomato seed oil improved recovery of lycopene from 18–56 %	Machmudah et al. (2012)
Lycopene	Tomato juice	CO_2 T = 313/353 K; P = 35 MPa	76.7 %	Effect of temperature and pressure on extraction efficiency. Identification and quantification of lycopene on the active fractions, yield and antioxidant capacity	Egydio et al. (2010)
Lycopene	Tomato skins	CO_2 T = 373 K; P = 40 MPa	3.125 mg/100 g	Increased bioavailability due to the generation of cis-isomers; antioxidant activity was evaluated	Yi et al. (2009)
Trans-Lycopene	Tomato skins	CO_2 + 14 % EtOH T = 335 K; P = 45 MPa	33 %	Increased yield was observed at higher temperatures	Kassama et al. (2008)
Lycopene	Tomato skins	CO_2 T = 373 K; P = 40 MPa	118 mg/100 g 94 %	Lycopene was extracted from tomato skin with negligible degradation under optimal conditions	Topal et al. (2006)
Ultrasound-assisted extraction					
Lycopene	Tomato peel	1:1 Petroleum ether and acetone, 328 K, 5–25 min, 460 W/cm^2	60 mg/100 g	Sonication significantly enhanced biocatalytic extraction of tomato-peel lycopene Antioxidant activity was evaluated. Extracted lycopene retained structural integrity and DPPH scavenging potency	Konwarh et al. (2012)

(continued)

Table 1.6 (continued)

Extracted compound(s)	Source/raw material	Extraction conditions[a]	Yield (mg/100 g)/ Recovery[b] (%)	Observations	Reference
Lycopene	Tomato	*n*-Hexane, acetone, EtOH; 45.6 min, 321 K, 74.4:1 (v/w)	76 %	UAE enhanced the extraction yield of lycopene, and at the same time minimized the degradation and isomerization of lycopene	Eh and Teoh (2012)
Lycopene	Tomato paste	Ethyl acetate, 29.1 min, 369 K, solvent to tomato paste 8.0:1 (v/w)	89.4 %	The percentage of lycopene recovered was higher when using ultrasonic- and microwave-assisted extraction (UMAE)	Lianfu and Zelong (2008)

EtOH Ethanol; *MeOH* Methanol; *nd* not determined
[a]selected optimal conditions for maximum recovery
[b]when compared with conventional extractions techniques

Table 1.7 Extraction of carotenoids from selected microorganisms and marine organisms by SC-CO_2, UAE, MAE and PLE

Extracted compounds	Source/raw material	Extraction conditions[a]	Yield (mg/100 g)/ Recovery[b] (%)	Reference
Supercritical CO_2 extraction				
Astaxanthin	*Phaffia rhodozyma*	CO_2 + EtOH (15 %) T = 313 K; P = 50 MPa	90 %	Lim et al. (2002)
Astaxanthin	*Haematococcus pluvialis*	CO_2 + 10 % olive oil T = 343 K; 40 MPa	51 %	Krichnavaruk et al. (2008)
Total carotenoids	*Chlorella vulgaris*	CO_2 T = 313/328 K; P = 20–35 MPa	≈50 mg/100 g	Mendes et al. (2003)
Astaxanthin	*Krill*	CO_2 T = 313–353 K; P = 24.4 MPa	nd	Yamaguchi et al. (1986)
Total carotenoids (mainly astaxanthin)	*Crustaceans*	CO_2 + EtOH (0-20 %) T = 313–333 K; P = 20–35 MPa	50 %	López et al. (2004)
β-Carotene	*Scenedesmus almeriensis*	CO_2 T = 333 K; P = 40 MPa	53 %	Macias-Sanchez et al. (2010)
Lutein	*Chlorella vulgaris*	CO_2 + EtOH T = 313 K; P = 40 MPa	70 %	Ruen-ngam et al. (2012)
Carotenoids (canthaxanthin and astaxanthin)	*Chlorella vulgaris*	CO_2 + EtOH T = 313 K; P = 30 MPa	246 mg/100 g 100 %	Palavra et al. (2011)
Astaxanthin	*Monoraphidium* sp. GK12	CO_2 + EtOH T = 303 K; P = 20 MPa	90 %	Fujii (2012)
Ultrasound assisted extraction				
β-Carotene	*Spirulina platensis*	*n*-Heptane T = 303 K;167 W/cm^2; 8 min	47 %	Dey and Rathod (2013)

(continued)

Table 1.7 (continued)

Extracted compounds	Source/raw material	Extraction conditions[a]	Yield (mg/100 g)/ Recovery[b] (%)	Reference
Microwave assisted extraction				
β-carotene Fucoxanthin	*Dunaliella tertiolecta* and *Cylindrotheca closterium*	Acetone T = 329 K; 50 W; 3–5 min	Hot soaking: 9.31 ± 0.44 mg/g	Pasquet et al. (2011)
Pressurized liquid extraction				
Carotenoids (astaxanthin, lutein)	*Haematococcus pluvialis*	EtOH T = 473 K; P = 10.34 MPa; 20 min	nd	Jaime et al. (2010)
Carotenoids (β-carotene, lutein, violaxanthin and neoxanthin)	*Phormidium*	EtOH T = 423 K; P = 10 MPa; 20 min	nd	Rodriguez-Meizoso et al. (2010)
Fucoxanthin	*Eisenia bicyclis*	90 % EtOH + 10 % water T = 383 K; P = 10 MPa; 5 min	42 mg/100 g	Shang et al. (2011)
Astaxanthin Lutein	*Haematococcus pluvialis and Dunaliella salina*	Acetone T = 313 K; P = 10 MPa two 5-min extraction cycles	Total astaxanthin: 950 mg/100 g Lutein 90 mg/100 g	Denery et al. (2004)
Zeaxanthin	*Chlorella elipsoidea*	EtOH T = 388 K P = 10 MPa; 23.3 min	428 mg/100 g	Koo et al. (2012)

EtOH Ethanol; *nd* not determined

[a]selected optimal conditions for maximum recovery

[b]when compared with conventional extractions techniques

applications (Reverchon and De Marco 2006). Vegetables oils have also been used as potential modifiers for the extraction of carotenoids (Longo et al. 2012).

SCFE of lycopene, a carotenoid with relevant antioxidant activity and protective effects against coronary heart disease and cancer, was performed using pure CO_2 with the modifiers (co-solvents) ethanol, olive oil, and hazelnut oil (Ciurlia et al. 2009; Perretti et al. 2013; Vasapollo et al. 2004; Shi et al. 2009; Baysal et al. 2000), and high recovery yields were obtained. Ultrasound-assisted extraction with organic solvents (petroleum ether, acetone, *n*-hexane, ethanol, and ethyl acetate) has also been explored (Konwarh et al. 2012; Eh and Teoh 2012; Lianfu and Zelong 2008). Table 1.6 summarizes studies involving lycopene extraction from tomato and tomato by-products.

In other works, astaxanthin, β-carotene, and other carotenoids have been extracted by SC-CO, MAE, ultrasonic-assisted extraction (UAE), and PLE from microorganisms and small marine animals that produce high levels of carotenoids. These studies are summarized in Table 1.7, and extraction yields, when available, are presented.

1.4.2 Fatty Acids

Several researchers have investigated SC-CO_2 extraction of oils rich in ω-3 fatty acids, and have demonstrated that this technology has a negligible environmental impact, and is thus also a potential tool for functional foods and nutraceuticals applications. For example, Cheung et al. (1998a) compared the yields of ω-3 fatty acids extracted from brown seaweed (*Sargassum hemiphyllum*) using SC-CO_2 (P = 24.1–37.9 MPa; T = 313/323 K) versus Soxhlet extraction with chloroform/methanol as control. Extraction conditions of 37.9 MPa/313 K and 37.9 MPa/323 K gave the highest lipid yield, which was comparable to that of the Soxhlet method (Fig. 1.4), and the highest concentrations of total and individual ω-3 fatty acids, which were significantly higher than with solvent extraction (Fig. 1.5).

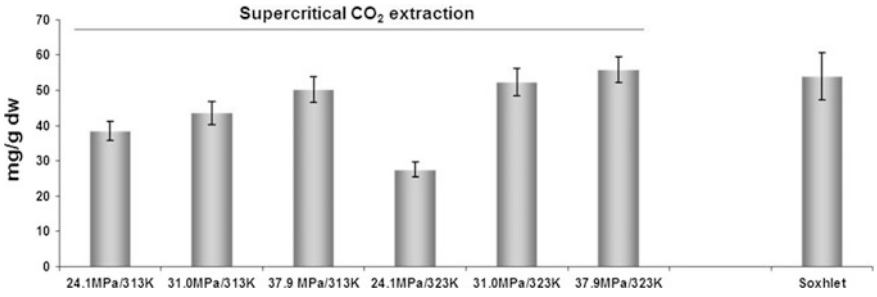

Fig. 1.4 Original lipid extracted (mg/g of dry weight) from *S. hemiphyllum* using SC-CO_2 and Soxhlet solvent extraction (Cheung et al. 1998b)

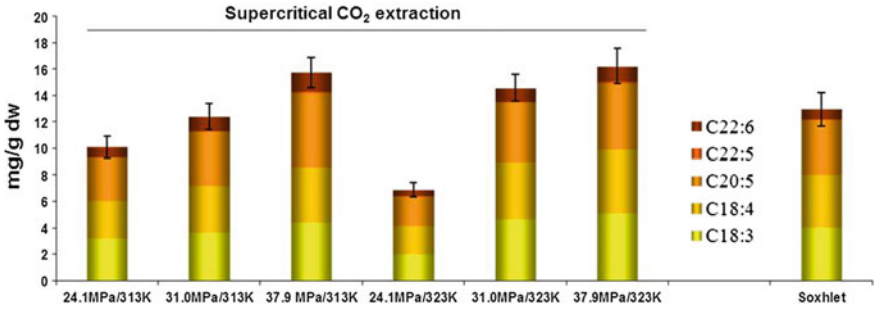

Fig. 1.5 Extraction yield of ω-3 fatty acids (mg/g of dry weight) from *S. hemiphyllum* using CO_2 and Soxhlet solvent extraction (Cheung et al. 1998b)

Amiguet et al. (2012) recently investigated the potential of northern shrimp by-products as a source of omega-3 polyunsaturated fatty acids (ω-3 PUFAs). Supercritical CO_2 extraction (SCFE) of shrimp by-products, at 35 MPa and 313 K, generated a deep red oil, rich in ω-3 PUFAs, specifically 7.8 ± 0.06 % eicosapentaenoic acid (EPA) and 8.0 ± 0.07 % docosahexaenoic acid DHA).

The SCFE of the omega-3-rich oil contained in hake by-products of the fish industry were studied by Rubio-Rodriguez et al. (2008) and the results are presented in Fig. 1.6. Extraction conditions of 25 MPa/313 K gave the highest lipid yield, which was comparable to that of the Soxhlet method (Fig. 1.6), and the highest concentrations of total fatty acids (FA), monounsaturated FA (MUFAs), polyunsaturated FA (PUFAs), EPA + DHA, ω-3, and ω-6 fatty acids, which were significantly higher than with the solvent extraction.

High-value seed oils are those that contain one or more PUFAs with desirable bioactivity. The most commonly extracted oils are those rich in γ-linoleic acid

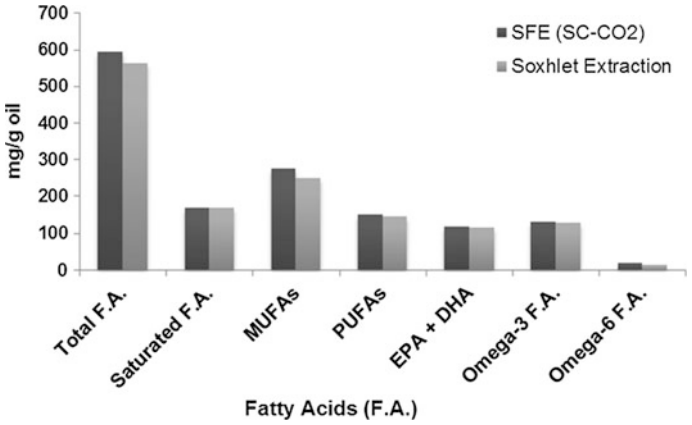

Fig. 1.6 Fatty acid profile of hake oil extracted with SC-CO_2 and with hexane as determined by AOAC method (Rubio-Rodríguez et al. 2008)

(GLA or C18:3 ω-3) and α-linoleic acid (ALA or C18:3 ω-6). They are traditionally produced by the extraction of hexane from ground seeds. Despite the efficiency of this extraction method, however, solvent elimination after extraction and possible thermal degradation of the oil represent major problems. Therefore, several authors have proposed the substitution of the traditional process with SC-CO_2 extraction of oil from seeds. In fact, the extraction of these oils by SC-CO_2 has reached the commercial stage, and the most commonly studied seeds are evening primrose, borage, blackcurrant, kiwifruit, hemp, and rose hip (Catchpole et al. 2009).

Wang et al. (2011) recently studied the fatty acid composition of tea (*Camellia sinensis* L.*)* seed oil extracted using optimized SC-CO_2. The objectives of this study were to employ SC-CO_2 to extract oil from tea seed and to determine the fatty acid composition of the extracted oil and its antioxidant activity. The authors then compared the extracts obtained by SC-CO_2 and by Soxhlet extraction. The total yield of tea seed oil by Soxhlet extraction was 25.3 ± 1.0 %, significantly lower than that (29.2 ± 0.6 %) using SC-CO_2 under optimal conditions.

The fatty acid (FA) profiles of tea seed oil extracted by SC-CO_2 and Soxhlet extraction (SE) were analysed by GC, and the results were similar (Fig. 1.7).

The tea seed oil extracted by SC-CO_2 showed much stronger DPPH radical scavenging ability than the oil extracted by Soxhlet.

Table 1.8 presents additional studies that explored the use of SC-CO_2 technology to recover fatty acids from plants, fish, algae, and fruits. non-conventional extraction techniques, namely MAE, UAE, and PLE, with hexane, ethanol, water, and acetyl lactate have been revealed as alternative technologies for isolating fatty acids and fatty acid methyl esters (FAME) from natural sources (Table 1.8).

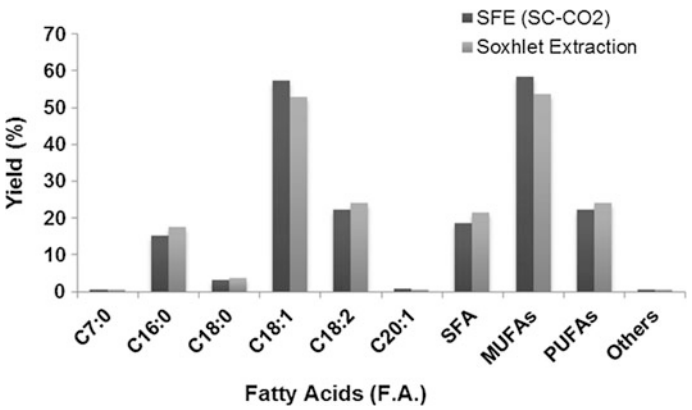

Fig. 1.7 Comparison of main fatty acid content of tea seed oil by different methods [adapted from (Wang et al. 2011)]

Table 1.8 Extraction of Fatty-acids by SC-CO_2, UAE, MAE and PLE

Extracted compounds	Source/raw material	Processing conditions[a]	Yield (mg/100 g)/ Recovery[b](%)	Reference
Supercritical CO_2 extraction[c]				
γ-Linolenic	*Arthrospira platensis*	CO_2 + EtOH T = 313 K; P = 30 MPa	35.3 %	Golmakani et al. (2012)
Fatty acids (PUFAs)	*Boletus edulis* (mushrooms)	CO_2 T = 313 K; P = 23.8 MPa	41–45 %	Vidovic et al. (2011)
Fatty acids (ω-3 PUFA, DHA, EPA)	Corn and fish oils	CO_2 T = 313; P = 16 MPa	nd	Weber et al. (2008)
Fatty acids (unsaturated)	Peach kernels	CO_2 T = 323 K; P = 30 MPa	91.5 %[d]	Mezzomo et al. (2010)
DHA	*Schizochytrium limacinum*	CO_2 + EtOH T = 313 K; P = 35 MPa	9.3 g/100 g	Tang et al. (2011)
Fatty acids (unsaturated)	Brazilian red-spotted shrimp waste	CO_2 + 15 % EtOH T = 323 K; P = 30 MPa	95.3 %	Sanchez-Camargo et al. (2012)
PUFAs	Brazilian red-spotted shrimp waste	CO_2 T = 323 K; P = 30 MPa	61.36 %	Sanchez-Camargo et al. (2011)
PUFAs	Fish by-product	CO_2 T = 333 K; P = 50 MPa	nd	Fiori et al. (2012)
ω-3 and ω-6 PUFAs	Fish oil	CO_2 T = 306/313 K; P = 20 MPa	nd	Lopes et al. (2012)
ω-3 and ω-6 PUFAs	Northern shrimp by-products	CO_2 T = 313 K; P = 35 MPa	nd	Amiguet et al. (2012)
PUFAs	Sea urchin gonad	CO_2 T = 323 K; P = 28 MPa	17.9 g/100 g	Zhu et al. (2010)
ω-3 and ω-6 PUFAs	Shellfish by-products	CO_2 T = 323 K; P = 28 MPa	17.8 g/100 g	Zhou et al. (2012)
Total fatty acids	Broccoli leaves	CO_2 T = 333 K; P = 30 MPa	1 g/100 g	Arnaiz et al. (2011)
PUFAs	Hemp seeds	CO_2 T = 313 K; P = 30 MPa	18 g/100 g	Da Porto et al. (2012)
PUFAs	Pomegranate seed oil	CO_2 T = 308 K; P = 15–30 MPa	nd[d]	Liu et al. (2012)
PUFAs	Sesame	CO_2 + EtOH T = 308–338 K; P = 20 MPa	Palmitic- 3.0 g/100 g; Stearic- 1.8 g/100 g; Oleic- 10.6 g/100 g; Linoleic- 10.9 g/100 g	Carvalho et al. (2012)

(continued)

Table 1.8 (continued)

Extracted compounds	Source/raw material	Processing conditions[a]	Yield (mg/100 g)/ Recovery[b](%)	Reference
Ultrasound-assisted extraction				
Fatty acids	Algae: *Nannochloropsis oculata*	Solvent-free, 1000 W, 30 min., 5 % dry weight;	EPA-62 mg/100 g	Adam et al. (2012)
Microwave-Assisted Extraction				
Fatty acids	Fish wastes from mackerel fillet and cod liver	Acetate:cyclohexane, 1:1 v/v, 75 W, 30 min, 2450 MHz	5.6 g/100 g mackerel and 62.6 g/100 g cod liver	Batista et al. (2001)
Pressurized liquid extraction				
Fatty acids	Algae: *Himanthalia elongata* and *Synechocystis* sp.	Hexane, EtOH and water, 323–473 K, 20 min	nd[d]	Plaza et al. (2010b)
Fatty acids	*Arthrospira platensis* (Spirulina)	EtOH:ethyl lactate (50:50), 453 K, 20 MPa, 15 min	68.3 %	Golmakani et al. (2012)

EtOH Ethanol; *nd* not determined
[a]selected optimal conditions for maximum recovery
[b]when compared with conventional extractions techniques
[c]studies performed between 2008 and 2013
[d]Antioxidant activity of extracts was evaluated

1.4.3 Tocopherols and Tocotrienols

The extraction of vitamin E from natural sources has attracted increasing interest due to the high antioxidant activity associated with this family of compounds. Moreover, natural vitamin E has been demonstrated to be more effective than a synthetic form (Friedrich 1987). Several natural matrices have been used as sources of vitamin E and tocopherols, and SCFE has been highlighted as one of the most promising methodologies for recovery of these compounds.

Wheat germ is an important source of vitamin E, and some studies have confirmed that SCFE (with CO_2) can achieve extraction yields for tocopherols similar to traditional hexane extraction (Molero and Martinez de la Ossa, 2000). In particular, Ge et al. (2002a) demonstrated that the extract derived from SCFE using the best operating conditions (P = 15 MPa; T = 213 K; CO_2 flow rate 1.5 L/min) presented higher quality (free fatty acids, 12.4 %; tocopherol content, 416.7 mg tocopherol/g wheat germ oil). Similarly another study performed with wheat germ showed higher extracted amounts of vitamin E and its isomers than those obtained using traditional methods (with *n*-hexane or chloroform/methanol mixtures) (Fig. 1.8). Thus, the extraction process using CO_2 may be economically competitive with conventional processes, as it shortens the oil refinement steps significantly and avoids the solvent distillation stage, both of which are very costly in terms of energy consumption.

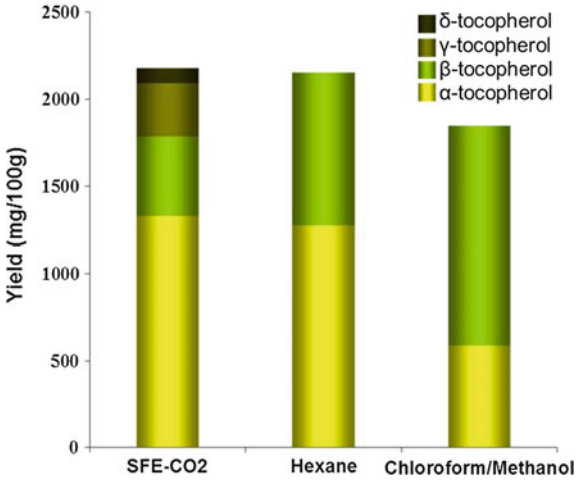

Fig. 1.8 Yield comparison of vitamin E and its isomers extracted by SCFE-CO_2 (P = 27.6 MPa; T = 313 K) with those by conventional solvent extraction (Ge et al. 2002b)

Table 1.9 summarizes published works regarding the extraction of tocopherols through SC-CO_2 and other non-conventional extraction techniques, namely MAE, UAE, and PLE.

1.4.4 Phytosterols

Plant-derived sterols in tissues and oil seeds can be isolated by solvent extraction with hexane, chloroform, diethyl ether, and acetone. However, these extraction procedures require large amounts of organic solvents, which are often expensive and potentially harmful. Extraction with supercritical carbon dioxide fluid constitutes an alternative that is advantageous, at least in terms of environmental impact (Lu et al. 2007).

For instance, King and Dunford (2002a) described a two-step supercritical fluid fractionation (SFF) method for obtaining phytosterol-enriched triglyceride fractions from rice bran and soybean oil deodorizer distillates (DD). The method comprised an extraction at 13.6 MPa and 318 K as a first step in order to remove the free fatty acids (not desirable in edible oils), while in a second step, the sterol ester-enriched triglyceride fractions were collected at 20.4 MPa and 353 K. With this method it was possible to obtain oil fractions with ~20 and 30 % (w/w) sterol content from rice bran oil and soybean oil DD, respectively, with relatively low free fatty acid content (Fig. 1.9) (King and Dunford 2002b).

The two-step SFF method demonstrated improvement over conventional methods used to obtain phytosterol-enriched products.

Huang et al. (2007) extracted β–sitosterol, stigmasterol, and ergosterol from *Anoectochilus* roxburghii. Under optimal conditions, i.e., pressure of 25 MPa, temperature of 318 K, and using ethanol as modifier, the concentrations of

Table 1.9 Extraction of tocopherols and tocotrienols by SC-CO_2, UAE, MAE and PLE

Extracted compounds	Source/raw-material	Processing conditions[a]	Yield (mg/g)/ Recovery[b] (%)	Reference
Supercritical CO_2 extraction				
Tocopherols	Sea buckthorn *(Hippophae rhamnoides)*	CO_2 T = 308 K; P = 40 MPa	77.2 %[c]	Kagliwal et al. (2011)
Tocopherols	Pomegranate seed oil	CO_2 T = 323 K; P = 45 MPa	0.43 mg/g[c]	Liu et al. (2012)
α-Tocopherol β-Tocopherol	Wheat bran	CO_2 T = 333 K; P = 30 MPa	0.03 mg/g 0.02 mg/g	Kwon et al. (2010)
α-Tocopherol γ-Tocopherol	Okra seed	CO_2 + EtOH T = 323 K; P = 45 MPa	0.15 mg/g 0.41 mg/g	Andrass et al. (2005)
α-Tocopherol β-Tocopherol γ-Tocopherol δ-Tocopherol	Wheat germ	CO_2 T = 313 K; P = 27 MPa	1.33 mg/g 0.46 mg/g 0.30 mg/g 0.09 mg/g	Ge et al. (2002a)
Pressurized liquid extraction				
Tocopherols	Palm oil	MeOH, acetonitrile, T = 323 K; P = 11 MPa 5 min	0.25 mg/g	Delgado-Zamarreno et al. (2009b)
Tocopherols	*Piper gaudichaudianum* Kunth	EtOH and petroleum ether T = 303 K; ethanol: 30 min; petroleum ether: 180 min	EtOH: 0.09 mg/g Petroleum ether: 0.02 mg/g	Peres et al. (2006)
Microwave-assisted extraction				
Tocopherols	Rice bran oil	Isopropanol and hexane, T = 393 K; solvent-to-rice bran ratio of 3:1 (w/w)	Isopropanol: 0.023 mg/g Hexane: 0.029 mg/g[c]	Zigoneanu et al. (2008)
Tocopherols	Wheat bran	MeOH T = 333–393 K; 500 W; 20 min	0.0195 mg/g[c]	Oufnac et al. (2007)
Ultrasound-assisted extraction				
Tocopherols	*Piper gaudichaudianum* Kunth	EtOH and petroleum ether T = 303 K; EtOH: 30 min; petroleum ether: 180 min	EtOH: 0.01 mg/g Petroleum ether: 0.0013 mg/g	Peres et al. (2006)

EtOH Ethanol; *MeOH* Methanol; *nd* not determined

[a]selected optimal conditions for maximum recovery

[b]when compared with conventional extractions techniques

[c]Antioxidant activity of extracts was evaluated

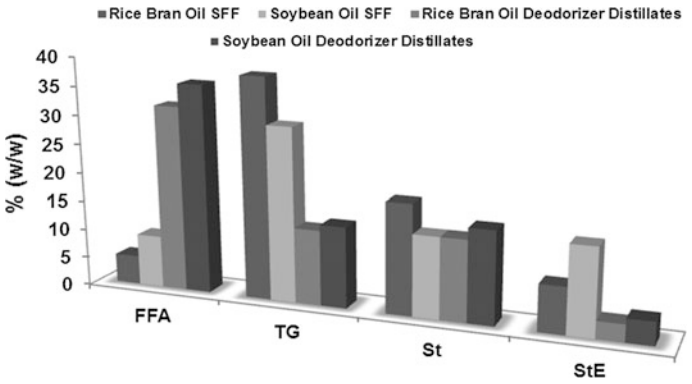

Fig. 1.9 Comparison of sterol and lipid composition of final products obtained using a two-step SFF method (rice bran oil SFF and soybean oil SFF) with rice bran oils DD and soybean oil DD as feeds of SFF (King and Dunford, 2002a)

β-sitosterol, stigmasterol, and ergosterol in the extract were found to be 2.89, 3.56, and 2.96 (g/g %), respectively (Fig. 1.10).

The concentrations of β-sitosterol, stigmasterol, and ergosterol in the extract were 4.7, 4.0 and 4.0 times higher, respectively, when SCFE was used compared with Soxhlet extraction. SCFE produced higher yields of sterols than did Soxhlet extraction.

Table 1.10 presents published works involving the extraction of phytosterols through SC-CO_2, MAE, UAE, and PLE.

1.4.5 Squalene

The importance of squalene in the cosmetic and pharmaceutical industries has driven the development of new technologies, such as counter-current SCFE, for the

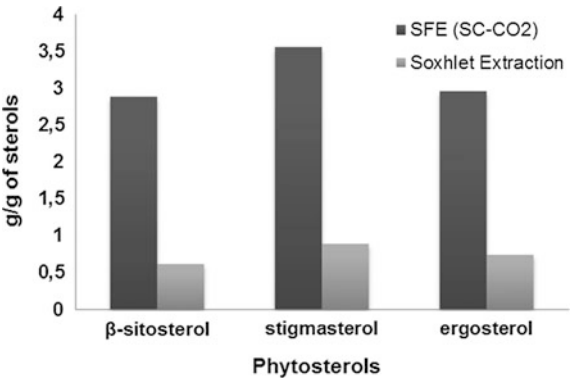

Fig. 1.10 Comparison of concentration of sterols obtained by SCFE and Soxhlet extraction [adapted from (Huang et al. 2007)]

Table 1.10 Extraction of phytosterols by SC-CO_2, UAE, MAE and PLE

Extracted compounds	Source/raw material	Processing conditions[a]	Yield (mg/g)/ recovery[b](%)	Reference
Supercritical CO_2 extraction				
Phytosterols	*Kalahari melon* seed	CO_2 T = 313 K; P = 30 MPa	0.064 mg/g	Nyam et al. (2011)
Sitosterols	Tomato by-products	CO_2 T = 353 K; P = 38 MPa	0.06 mg/g	Vagi et al. (2007)
β-Sitosterol Stigmasterol	*A. roxburghii*	CO_2 + ethanol T = 318 K; P = 25 MPa	1.06 mg/g 1.19 mg/g	Huang et al. (2007)
β-Sitosterol	Roasted loquat seed	CO_2 T = 353 K; P = 30 MPa	≈75 %	Kawahito et al. (2008)
Phytosterols	Sea buckthorn (*Hippophae rhamnoides* L.)	CO_2 T = 323 K; P = 51.7 MPa	nd	Li et al. (2007)
Campesterol Stigmasterol β-Sitosterol β-Amyrin	Lotus bee pollen (*Nelumbo nucifera* Gaertn)	CO_2 T = 323 K; P = 38.2 MPa	0.49 mg/g	Xu et al. (2011b)
Phytosterols	Roselle seed (*Hibiscus sabdariffa* L.)	CO_2 + ethanol T = 313 K; P = 40 MPa	0.040 mg/g	Nyam et al. (2010)
β-Sitosterol	Sea buckthorn seed (*Hippophae rhamnoides* L)	CO_2 T = 313 K; P = 15 MPa	0.31 mg/g	Sajfrtova et al. (2010)
Pressurized liquid extraction				
β-Sitosterol	Seeds and nuts	MeOH, 50°C; 11 MPa and two 5 min cycles	Almond- 1.16 mg/g Pistachio-1.85 mg/g	Delgado-Zamarreno et al. (2009a)
Fucosterol	*Himanthalia elongate*	EtOH, 100°C, 10 MPa, 20 min	nd[c]	Santoyo et al. (2011)
Stigmasterol β-Sitosterol	*Piper gaudichaudianum* Kunth	EtOH and petroleum ether, 303 K, EtOH: 30 min; petroleum ether: 180 min.	Stigmasterol: EtOH-0.06 mg/g Petroleum ether-0.05 mg/g β-Sitosterol: EtOH-0.02 mg/g Petroleum ether-0.01 mg/g	Peres et al. (2006)
Ultrasound-assisted extraction				
Stigmasterol β-Sitosterol	*Piper gaudichaudianum* Kunth	EtOH and petroleum ether, 303 K, ethanol: 30 min; petroleum ether: 180 min.	Stigmasterol: EtOH-0.005 mg/g Petroleum ether-0.004 mg/g β-Sitosterol: EtOH-0.010 mg/g Petroleum ether-0.0009 mg/g	Peres et al. (2006)
Microwave-assisted extraction				
Fucosterol	Marine algae	Ethanolic potassium hydroxide, 30:1 (liquid:solid ratio), 343 K, 300 W, 30 min	1.15 mg/g	Xiao et al. (2013)

[a]selected optimal conditions for maximum recovery
EtOH Ethanol; *MeOH* Methanol; *nd* not determined
[b]when compared with conventional extractions techniques
[c]Antioxidant activity of extracts was evaluated

Table 1.11 Extraction of squalene from animal and vegetable sources

Source	Squalene concentration (%w/w)		Reference
	Raw material (%)	SCF extract (%)	
Shark liver oil	55	92–99	Catchpole et al. (1997)
Olive oil by-product	52	90	Vasquez et al. (2007)
Palm fatty acid distillate (PFAD)	50	95	Al-Darmaki et al. (2012)

purification of squalene from various sources. The primary source of squalene is shark liver oil, but it can also be found in olive oil by-products.

The recovery of squalene from shark liver oil by SCFE has been reported in the literature (Catchpole et al. 2000a, b). This raw material also contains many triacylglycerols, alkoxyglycerols, sterol esters, and pristine squalene. Therefore, extraction process conditions are needed that allow the highest solubility of only squalene, in order to maximize the purity and yield of the extract. This is also the case when olive oil DD containing free fatty acids and methyl and ethyl esters are used as raw materials.

Al-Darmaki et al. (2012) recently studied the extraction and recovery of squalene using SC-CO_2 as solvent from palm fatty acid distillate (PFAD). The process was carried out on a counter-current glass bead-packed column. The operating conditions investigated were pressure and temperature, which varied from 10 to 20 MPa and from 313 to 353 K, respectively (Al-Darmaki et al. 2012).

Table 1.11 presents three studies related to successful isolation of squalene-rich extracts from shark liver oil and olive oil by-products.

1.4.6 Essential Oils

Essential oils have become a target for the recovery of natural bioactive substances. According to Fornari et al. (2012), since 2000, nearly 4000 articles have been published in which "essential oil" or "volatile oil" appeared as keyword, and around 3000 of these also included the word "bioactive" or "bioactivity" in the article text (Fornari et al. 2012). Moreover, essential oils and their extraction have been reviewed extensively (Moyler 1993; Chen and Spiro 1994; Kerrola 1995; Reverchon 1997; Burt 2004; Lucchesi et al. 2004; Edris 2007; Mustafa and Turner 2011; Mason et al. 2011; Xu et al. 2011a; Sovová 2012; Abad et al. 2012; Fornari et al. 2012; Kokolakis and Golfinopoulos 2013; Capuzzo et al. 2013). For this reason, an overview of selected publications regarding the extraction of essential oils using different methodologies is presented instead of a resume table.

In industrial practice, essential oils are obtained by steam distillation; in laboratory practice, steam distillation and hydro-distillation are used. However, processing temperature is a drawback in the extraction of thermally labile compounds (Pereira and Meireles 2010). In order to overcome this, SCFs have been used

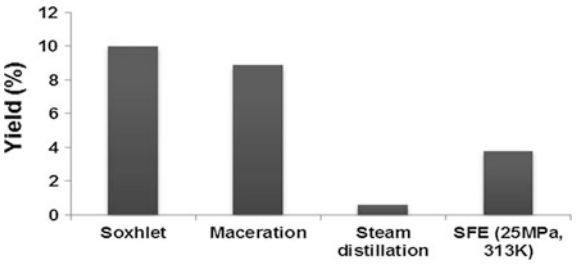

Fig. 1.11 Extraction Yield (%) of chamomile flower head with SCFE and conventional methods [adapted from (Kotnik et al. 2007)]

successfully for the recovery of these substances, and in some cases, comparisons with conventional methods have been performed.

Chamomile flower heads, which are widely used in the cosmetic, pharmaceutical, and food industries due to their biological activity (anti-spasmodic, anti-inflammatory, and antimicrobial properties), were extracted using SC-CO_2 technology (Kotnik et al. 2007). Various pressures (10, 15, and 25 MPa) and temperatures (303 and 313 K) were assessed, with the highest extraction yield achieved at 25 MPa and 313 K. The results were further compared with those obtained using Soxhlet extraction, steam distillation, and maceration (Fig. 1.11), and SC-CO_2 was found to offer considerable advantages over the others. Although the yield was lower, the extract obtained with SC-CO_2 had much higher content of the active compounds matricine and bisabol (Fig. 1.12). In addition, using SC-CO_2, the matricine content was able to be increased almost threefold by using two-step separation procedures. It is also important to note that the process was successfully implemented at a pilot scale.

The same was observed in a study conducted by Moura et al. (2012), which evaluated SCFE of essential oils using ethanol and isopropyl alcohol as co-solvents. The global yield obtained with conventional techniques, including Soxhlet extraction, low-pressure solvent extraction (LPSE), ultrasound, and hydro-distillation, were compared with the global yield of SCFE (Moura et al. 2012). Essential oils were detected in extracts from all extraction methods used in this study (Fig. 1.13). Although higher global yields were obtained with conventional extraction methods, SCFE resulted in better recovery of essential oils.

In another work, three methods—hydro-distillation, PLE, and SCFE—were optimized and compared for the extraction of volatile compounds from *Cyperus*

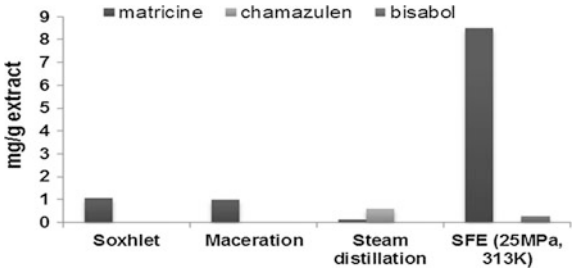

Fig. 1.12 Content of active components in chamomile extracts obtained by SCF extraction at 25 MPa, 313 K, with SCFE and conventional methods [adapted from (Kotnik et al. 2007)]

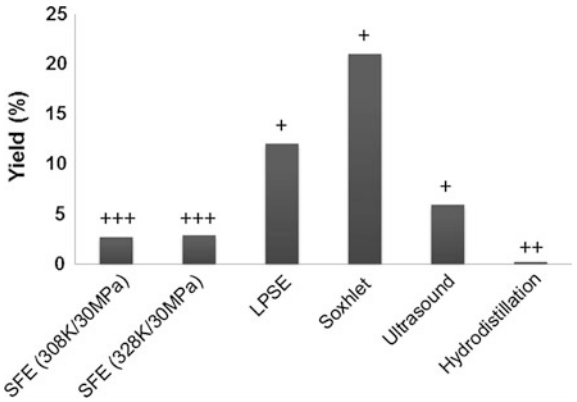

Fig. 1.13 Global yield and relative intensity of essential oils in extracts of *P. guajava* leaves from different extraction processes [adapted from (Moura et al. 2012)]

rotundus. The study showed the highest extraction efficiency with PLE, while SCFE had the best selectivity for the extraction of β-cyperone and α-cyperone (Tam et al. 2007).

The extraction of lavender essential oil was investigated using hydro-distillation, SC-CO_2, and hexane extraction techniques, and results were compared in terms of yield, chemical composition, and antimicrobial and antioxidant activity. SC-CO_2 produced a yield of 6.7 % (dry weight), which was comparable to that of solvent extraction (7.6 %) but significantly higher than that of hydro-distillation (4.6 %). The chemical composition of the oils showed considerable variation among extraction methods. Hexane extraction produced oils with the presence of waxes, colour pigments, and albuminous materials with semi-solid consistency, while hydro-distillation extracts showed evidence of thermal degradation. The SC-CO_2 extract had an aroma most closely resembling the starting material, showed negligible thermal degradation, and exhibited significantly higher antioxidant activity than the hydro-distillation and hexane extracts. Antimicrobial activity was higher in oils produced by SC-CO_2 and hydro-distillation than by hexane extraction. The results of this study demonstrate that SC-CO_2 is a promising technique for the extraction of lavender essential oil (Danh et al. 2013).

In a study by Okoh et al. (2010), *Rosmarinus officinalis* essential oil extracts obtained by hydro-distillation and microwave extraction were compared in terms of yield and antibacterial activity. Briefly, the total yield of the volatile fractions obtained through hydro-distillation and microwave extraction were 0.31 and 0.39 %, respectively. With regard to the minimum inhibitory concentration (MIC) of oils obtained by the two methods, results showed greater activity against microorganisms in the oil obtained by microwave extraction than that obtained by hydro-distillation. This observation was explained by the fact that the microwave-extracted oil contained more oxygenated compounds, and that this class of compounds has been proven to possess strong antibacterial and antifungal activity. This result shows that the composition of essential oils may vary depending on the method of extraction used (Okoh et al. 2010).

In a study by Herzi et al. (2013), the extraction of two species of *Eucalyptus* by SC-CO_2 and hydro-distillation were compared in terms of yield, chemical composition, and antioxidant activity. Superior yields were obtained using SC-CO_2. The antioxidant activity of the extracts was also assessed using the two methods, and promising radical scavenging activity was observed in the SC-CO_2 extracts. In this work, SC-CO_2 demonstrated important advantages compared to hydro-distillation, including faster extraction, improved yield, and extracts with high antioxidant quality (Herzi et al. 2013).

1.5 Process Optimization for Targeting Bioactivity

Over the last few years, special focus has been given to the design and optimization of extraction processes for the recovery of mixtures of bioactive molecules with potential health benefits, rather than the isolation of pure and single compounds. In this field, the recovery of anticancer-bioactive-rich fractions from natural sources has attracted the attention of the scientific community in their efforts to develop new natural chemotherapeutic agents. For this propose, high-pressure processes, including SCFE, were the most common recovery technologies applied. For example, in the work performed by Serra et al. (2010) a high-pressure fractionation process was optimized to isolate compounds with antiproliferative effects against colon cancer, including perillyl alcohol from sweet cherries ("Saco" variety). The methodology employed comprised a first step with SC-CO_2 followed by a second step using mixtures of CO_2 and ethanol. The effect of SC-CO_2 pre-treatment and the influence of the ethanol concentration in the solvent mixture composition was studied in relation to extract yield, phenolic content, and antiproliferative effects in human colon cancer cells. The product derived from CO_2:ethanol (90:10 v/v) extraction exhibited the highest antiproliferative activity, likely due to the presence of perillyl alcohol and high polyphenol content in the resulting extract (Fig. 1.14).

This methodology was later applied to other sweet cherry varieties, as well as to plum and peach pomaces, in order to evaluate its effectiveness on the isolation of bioactive fractions with anticancer properties from these natural sources (Silva 2013). Results showed that all extracts inhibited colon cancer cell growth and exhibited similar terpenes profiles (Fig. 1.15), evidencing the selectivity of this extraction methodology.

In a study performed by Vicente et al. (2013) SCFs technology was explored for producing rosemary extracts of different composition and their anticancer effects in human liver carcinoma cells. Several extraction conditions employed produced different extract composition in terms of carnosic acid and volatile compounds, namely 1,8 cineole, camphor, borneol, verbenone, and bornyl acetate. Results showed that the conditions P = 15 MPa, 5 % (w/w) of ethanol as co-solvent and t = 180 min obtained a fraction with an effective composition of carnosic acid and volatile compounds demonstrating the highest antiproliferative potential in cancer cells (Fig. 1.16). Among compounds, carnosic acid was shown to have a crucial

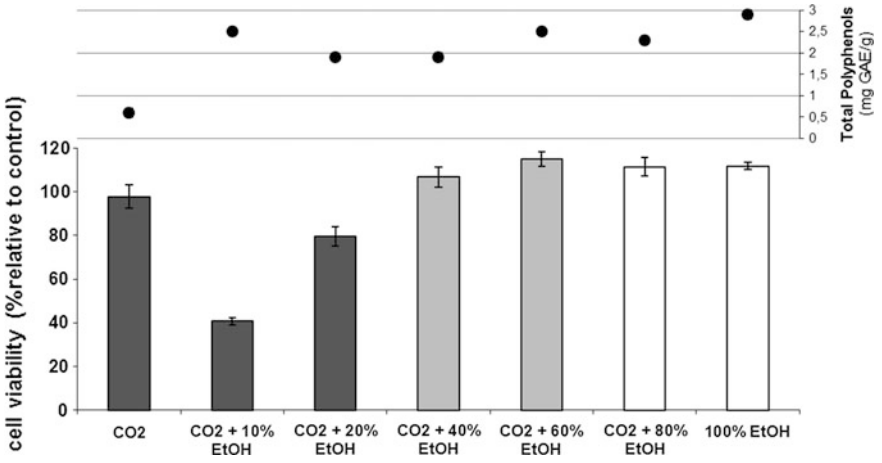

Fig. 1.14 Antiproliferative effect in HT29 colon cancer cell line of different cherry extracts produced by high-pressure technology (P = 250 bar, T = 323 K) (results obtained after 96 h of treatment with 0.5 mg/mL of extract). Comparison of bioactive effect with phenolic content (*filled circle*) and presence of perillyl alcohol (*dark filled bar*-well-defined zone detected; *light filled bar*-less-defined zone detected; *unfilled bar*-none detected) [adapted from Serra et al. (2010)]

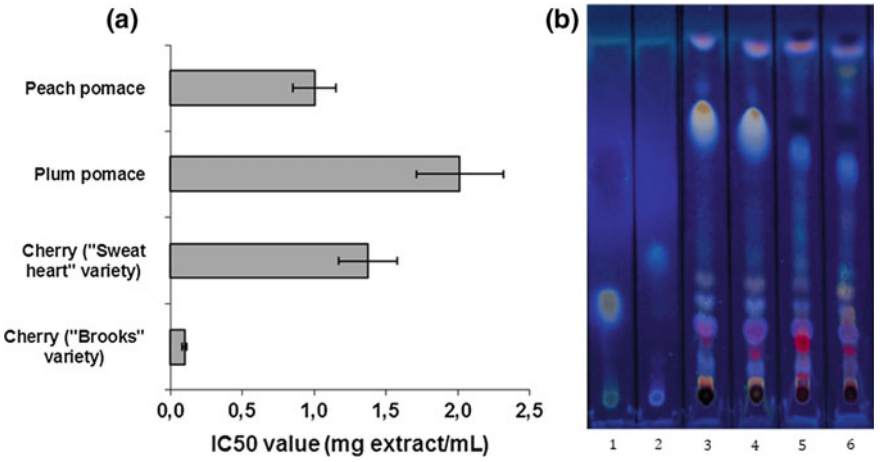

Fig. 1.15 Characterization of cherry, peach and plum extracts obtained using high-pressure technology (P = 250 bar, T = 323 K, CO_2 + EtOH 10 %). **a** Antiproliferative effect on HT29 cell line after 24 h of treatment- IC50 values; **b** Terpene profile detected by TLC (Legend: *1* perillyl alcohol standard; *2* Linalool standard; *3* Brucks cherry; *4* Sweet Hearth cherry; *5* plum; *6* peach) [adapted from Silva (2013)]

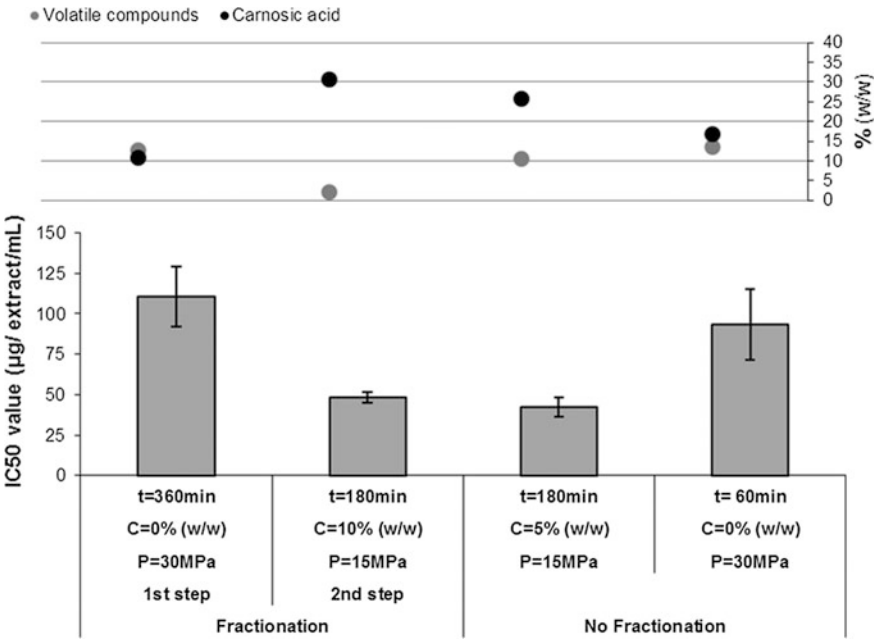

Fig. 1.16 Comparison of the antiproliferative effect in HepG2 cells of rosemary extracts obtained by supercritical fluid technology with composition in terms of carnosic acid and volatile compounds. Rosemary extracts were obtained using supercritical fluid technology under different pressure conditions and percentages of co-solvent (ethanol); (IC50 values were obtained after 48 h of treatment) (Vicente et al. 2013)

effect on growth inhibition conferred by rosemary extract. Moreover, other substances comprising the volatile oil fraction may synergize with the rosemary compound in its anti-tumoural action.

Together, these studies demonstrate the importance not only of developing methods for isolating individual compounds, but also of optimizing processes for separating fractions rich in synergistic mixtures of bioactivity.

1.6 Summary

Lipophilic compounds of various structures have shown promising bioactivity. In this chapter, conventional and new alternative techniques for the recovery and isolation of high-value lipophilic bioactives have been reviewed, with particular emphasis on supercritical fluid extraction.

References

Abad MJ, Bedoya LM, Apaza L, Bermejo P (2012) The *Artemisia L.* Genus: a review of bioactive essential oils. Molecules 17:2542–2566

Abboud J, Notario R (1999) Critical compilation of scale solvent parameters. Part I. Pure, non-hydrogen bond donor solvents. Pure Appl Chem 71:645–718

Adam F, Abert-Vian M, Peltier G, Chemat F (2012) "Solvent-free" ultrasound-assisted extraction of lipids from fresh microalgae cells: a green, clean and scalable process. Bioresour Technol 114:457–465

Adil IH, Cetin HI, Yener ME, Bayindirli A (2007) Subcritical (carbon dioxide plus ethanol) extraction of polyphenols from apple and peach pomaces, and determination of the antioxidant activities of the extracts. J Supercrit Fluids 43:55–63

Adil İH, Yener ME, Bayındırlı A (2008) Extraction of total phenolics of sour cherry pomace by high pressure solvent and subcritical fluid and determination of the antioxidant activities of the extracts. Sep Sci Technol 43:1091–1110

Ajila C, Brar S, Verma M, Tyagi R, Goudbout S, Valero J (2010) Extraction and analysis of polyphenols: recent trends. Crit Rev Biotechnol. Informa Healthcare

Al-Darmaki N, Lu T, Al-Duri B, Harris JB, Favre TLF, Bhaggan K, Santos RCD (2012) Isothermal and temperature gradient supercritical fluid extraction and fractionation of squalene from palm fatty acid distillate using compressed carbon dioxide. J Supercrit Fluids 61:108–114

Amiguet V, Kramp KL, Mao J, McRae C, Goulah A, Kimpe LE, Blais JM, Arnason JT (2012) Supercritical carbon dioxide extraction of polyunsaturated fatty acids from northern shrimp (Pandalus borealis Kreyer) processing by-products. Food Chem 130:853–858

Andıran F, Ayhan A, Tanyel FC, Abbasoğlu O, Sayek İ (2000) Regenerative capacities of normal and cirrhotic livers following 70 % hepatectomy in rats and the effect of α-Tocopherol on cirrhotic regeneration. J Surg Res 89:184–188

Andrass CD, Simanndi B, Orsi F, Lambrou C, Missopolinou-Tatala D, Panayiotou C, Domokos J, Doleschall F (2005) Supercritical carbon dioxide extraction of okra (*Hibiscus esculentus L*) seeds. J Sci Food Agric 85:1415–1419

Arnaiz E, Bernal J, Martin MT, Garcia-Viguera C, Bernal JL, Toribio L (2011) Supercritical fluid extraction of lipids from broccoli leaves. Eur J Lipid Sci Technol 113:479–486

Astorg P (1997) Food carotenoids and cancer prevention: an overview of current research. Trends Food Sci Technol 8:406–413

Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, Jahurul MHA, Ghafoor K, Norulaini NAN, Omar AKM (2013) Techniques for extraction of bioactive compounds from plant materials: a review. J Food Eng SI Extr Encapsul

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils—a review. Food Chem Toxicol 46:446–475

Batista A, Vetter W, Luckas B (2001) Use of focused open vessel microwave-assisted extraction as prelude for the determination of the fatty acid profile of fish—a comparison with results obtained after liquid-liquid extraction according to Bligh and Dyer. Eur Food Res Technol 212:377–384

Baysal T, Ersus S, Starmans DAJ (2000) Supercritical CO2 extraction of beta-carotene and lycopene from tomato paste waste. J Agric Food Chem. American Chemical Society

Bendich A (1994) Recent advances in clinical research involving carotenoids. Pure Appl Chem 66:1017–1024

Briganti S, Picardo M (2003) Antioxidant activity, lipid peroxidation and skin diseases. What's new. J Eur Acad Dermatol Venereol 17:663–669

Brunner G (2005a) Supercritical fluids: technology and application to food processing. J Food Eng IV Iberoamerican Congr Food Eng (CIBIA IV)

Brunner G (2005b) Supercritical fluids: technology and application to food processing. J Food Eng 67:21–33

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94:223–253

Burton-Freeman B, Reimers K (2011) Tomato consumption and health: emerging benefits. Am J Lifestyle Med 5:182–191

Cadoni E, de Giorgi MR, Medda E, Poma G (1999) Supercritical CO2 extraction of lycopene and beta-carotene from ripe tomatoes. Dyes Pigm 44:27–32

Capuzzo A, Maffei ME, Occhipinti A (2013) Supercritical fluid extraction of plant flavors and fragrances. Molecules 18:7194–7238

Carvalho RHR, Galvao EL, Barros JAC, Conceicao MM, Sousa EMBD (2012) Extraction, fatty acid profile and antioxidant activity of sesame extract (*Sesamum Indicum L.*). Braz J Chem Eng 29:409–420

Catchpole OJ, Von Kamp JC, Grey JB (1997) Extraction of squalene from shark liver oil in a packed column using supercritical carbon dioxide. Ind Eng Chem Res. American Chemical Society

Catchpole OJ, Grey JB, Noermark KA (2000a) Fractionation of fish oils using supercritical CO_2 and CO_2+ethanol mixtures. J Supercrit Fluids 19:25–37

Catchpole OJ, Simoes P, Grey JB, Nogueiro EMM, Carmelo PJ, Nunes da Ponte M (2000b) Fractionation of lipids in a static mixer and packed column using supercritical carbon dioxide. Ind Eng Chem Res. American Chemical Society

Catchpole OJ, Tallon SJ, Eltringham WE, Grey JB, Fenton KA, Vagi EM, Vyssotski MV, Mackenzie AN, Ryan J, Zhu Y (2009) The extraction and fractionation of specialty lipids using near critical fluids. J Supercrit Fluids 20th Year Anniversary Issue J Supercrit Fluids

Cavalcanti RN, Forster-Carneiro T, Gomes MTMS, Rostagno MA, Prado JM, Meireles MAA (2013) Chapter 1 uses and applications of extracts from natural sources. Nat Prod Extr Principles Appl. The Royal Society of Chemistry

Cavero S, Garcia-Risco MR, Marin FR, Jaime L, Santoyo S, Senorans FJ, Reglero G, Ibanez E (2006) Supercritical fluid extraction of antioxidant compounds from oregano: chemical and functional characterization via LC-MS and in vitro assays. J Supercrit Fluids 38:62–69

Cheah ELC, Chan LW, Heng PWS (2006) Supercritical carbon dioxide and its application in the extraction of active principles from plant materials. Asian J Pharm Sci 1:59–71

Chemat F, Tomao V, Virot M (2008) Ultrassound-assisted extraction in food analysis. In: OTLES S (ed) Handbook of food analysis instruments. CRC Press

Chen S, Spiro M (1994) Study of microwave extraction of essential oil constituents from plant materials. J Microw Power Electromagn Energy 29:231–241

Cheung PCK, Leung AYH, Ang PO (1998a) Comparison of supercritical carbon dioxide and soxhlet extraction of lipids from a brown seaweed, *Sargassum hemiphyllum* (Turn) C. Ag. J Agric Food Chem. American Chemical Society

Cheung PCK, Leung AYH, Ang PO (1998b) Comparison of supercritical carbon dioxide and soxhlet extraction of lipids from a brown seaweed, *Sargassum hemiphyllum* (Turn) C. Ag.†. J Agric Food Chem 46:4228–4232

Chew BP (1995) Antioxidant vitamins affect food animal immunity and health. J Nutr 125:1804S–1808S

Ciurlia L, Bleve M, Rescio L (2009) Supercritical carbon dioxide co-extraction of tomatoes (*Lycopersicum esculentum L.*) and hazelnuts (*Corylus avellana L.*): a new procedure in obtaining a source of natural lycopene. J Supercrit Fluids 49:338–344

Cunnane SC (2003) Problems with essential fatty acids: time for a new paradigm? Prog Lipid Res 42:544–568

da Porto C, Decorti D, Tubaro F (2012) Fatty acid composition and oxidation stability of hemp (*Cannabis sativa L.*) seed oil extracted by supercritical carbon dioxide. Ind Crops Prod 36:401–404

Daintree LS, Kordikowski A, York P (2008) Separation processes for organic molecules using SCF technologies. Adv Drug Delivery Rev Drug Delivery Appl Supercrit Fluid Technol

Danh L, Han L, Triet N, Zhao J, Mammucari R, Foster N (2013) Comparison of chemical composition, antioxidant and antimicrobial activity of lavender (*Lavandula angustifolia L.*)

essential oils extracted by supercritical CO_2, Hexane and hydrodistillation. Food Bioprocess Technol 6:3481–3489
de Paz E, Martin A, Duarte CMM, Cocero MJ (2012) Formulation of beta-carotene with poly-(e-caprolactones) by PGSS process. Powder Technol 217:77–83
Delgado-Zamarreno MM, Bustamante-Rangel M, Martinez-Pelarda D, Carabias-Martinez R (2009a) Analysis of beta-sitosterol in seeds and nuts using pressurized liquid extraction and liquid chromatography. Anal Sci 25:765–768
Delgado-Zamarreno MM, Bustamante-Rangel M, Sierra-Manzano S, Verdugo-Jara M, Carabias-Martinez R (2009b) Simultaneous extraction of tocotrienols and tocopherols from cereals using pressurized liquid extraction prior to LC determination. J Sep Sci 32:1430–1436
Denery JR, Dragull K, Tang CS, Li QX (2004) Pressurized fluid extraction of carotenoids from *Haematococcus pluvialis* and *Dunaliella salina* and kavalactones from *Piper methysticum*. Anal Chim Acta 501:175–181
Destandau E, Michel T, Elfakir C (2013) Chapter 4 microwave-assisted extraction. Nat Prod Extr Principles Appl. The Royal Society of Chemistry
Dey S, Rathod VK (2013) Ultrasound assisted extraction of beta-carotene from *Spirulina platensis*. Ultrason Sonochem 20:271–276
Díaz-Reinoso B, Moure A, Domínguez H, Parajó JC (2006) Supercritical CO_2 extraction and purification of compounds with antioxidant activity. J Agric Food Chem 54:2441–2469
Dunford NT, Irmak S, Jonnala R (2010) Pressurised solvent extraction of policosanol from wheat straw, germ and bran. Food Chem 119:1246–1249
Edris AE (2007) Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytotherapy Res 21:308–323
Egydio JA, Moraes AM, Rosa PTV (2010) Supercritical fluid extraction of lycopene from tomato juice and characterization of its antioxidation activity. J Supercrit Fluids 54:159–164
Eh AL, Teoh SG (2012) Novel modified ultrasonication technique for the extraction of lycopene from tomatoes. Ultrason Sonochem 19:151–159
Esclapez MD, Garcia-Perez JV, Mulet A, Carcel JA (2011) Ultrasound-assisted extraction of natural products. Food Eng Rev Food Eng Rev. Springer-Verlag
Fernandes P, Cabral JM (2007) Phytosterols: applications and recovery methods. Bioresour Technol 98:2335–2350
Fernandez-Ronco MP, De Lucas A, Rodriguez JF, Garcia MT, Gracia I (2013) New considerations in the economic evaluation of supercritical processes: Separation of bioactive compounds from multicomponent mixtures. The journal of supercritical fluids special issue—10th international symposium on supercritical fluids special issue—10th international symposium on supercritical fluids
Fiori L, Solana M, Tosi P, Manfrini M, Strim C, Guella G (2012) Lipid profiles of oil from trout (*Oncorhynchus mykiss*) heads, spines and viscera: trout by-products as a possible source of omega-3 lipids? Food Chem 134:1088–1095
Folmer BM, Barron D, Hughes E, Miguet L, Sanchez B, Heudi O, Rouvet M, Sagalowicz L, Callier P, Michel M, Williamson G (2009) Monocomponent hexa- and dodecaethylene glycol succinyl-tocopherol esters: self-assembly structures, cellular uptake and sensitivity to enzyme hydrolysis. Biochem Pharmacol 78:1464–1474
Fornari T, Vicente G, Vázquez E, García-Risco MR, Reglero G (2012) Isolation of essential oil from different plants and herbs by supercritical fluid extraction. J Chromatogr A 1250:34–48
Friedman M, Henika PR, Mandrell RE (2002) Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes*, and *Salmonella enterica*. J Food Prot® 65:1545–1560
Friedrich W (1987) Handbuch der vitamine. Urban & Schwarzenberg, Munchen
Fritsche K (2006) Fatty acids as modulators of the immune response. Annu Rev Nutr 26:45–73
Fujii K (2012) Process integration of supercritical carbon dioxide extraction and acid treatment for astaxanthin extraction from a vegetative microalga. Food Bioprod Process 90:762–766

Garcia C, Talarico L, Almeida N, Colombres S, Duschatzky C, Damonte E (2003) Virucidal activity of essential oils from aromatic plants of San Luis, Argentina. Phytotherapy Res 17:1073–1075

Ge Y, Yan H, Hui B, Ni Y, Wang S, Cai T (2002a) Extraction of natural vitamin E from wheat germ by supercritical carbon dioxide. J Agric Food Chem. American Chemical Society

Ge Y, Yan H, Hui B, Ni Y, Wang S, Cai T (2002b) Extraction of natural vitamin E from wheat germ by supercritical carbon dioxide. J Agric Food Chem 50:685–689

Gerster H (1993) Anticarcinogenic effect of common carotenoids. Internat J Vit Nutr Res J Vit Nutr Res

Geusens P, Wouters C, Nijs J, Jiang Y, Dequeker J (1994) Long-term effect of omega-3 fatty acid supplementation in active rheumatoid arthritis. Arthritis Rheum 37:824–829

Golmakani MT, Mendiola JA, Rezaei K, Ibanez E (2012) Expanded ethanol with CO_2 and pressurized ethyl lactate to obtain fractions enriched in γ-Linolenic Acid from Arthrospira platensis (Spirulina). J Supercrit Fluids 62:109–115

Haeger K (1974) Long-time treatment of intermittent claudication with vitamin E. Am J Clin Nutr 27:1179–1181

He HP, Cai Y, Sun M, Corke H (2001) Extraction and purification of squalene from amaranthus grain. J Agric Food Chem. American Chemical Society

Herrera MC, de Castro MD (2005) Ultrasound-assisted extraction of phenolic compounds from strawberries prior to liquid chromatographic separation and photodiode array ultraviolet detection. J Chromatogr A 1100:1–7

Herrero M, Cifuentes A, Ibanez E (2006a) Sub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae—a review. Food Chem 98:136–148

Herrero M, Cifuentes A, Ibanez E (2006b) Sub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae: a review. Food Chem 98:136–148

Herrero M, Mendiola JA, Cifuentes A, Ibanez E (2010) Supercritical fluid extraction: recent advances and applications. J Chromatogr A Extr Tech

Herzi N, Bouajila J, Camy S, Cazaux S, Romdhane M, Condoret JS (2013) Comparison between supercritical CO_2 extraction and hydrodistillation for two species of eucalyptus: yield, chemical composition, and antioxidant activity. J Food Sci 78:C667–C672

Hodge L, Salome C, Hughes J, Liu-Brennan D, Rimmer J, Allman M, Pang D, Armour C, Woolcock A (1998) Effect of dietary intake of omega-3 and omega-6 fatty acids on severity of asthma in children. Eur Respir J 11:361–365

Hooper L, Thompson RL, Harrison RA, Summerbell CD, Ness AR, Moore HJ, Worthington HV, Durrington PN, Higgins J, Capps NE (2006) Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. BMJ Brit Med J (Int Edn) 332

Huang L, Zhong T, Chen T, Ye Z, Chen G (2007) Identification of beta-sitosterol, stigmasterol and ergosterin in A. roxburghii using supercritical fluid extraction followed by liquid chromatography/atmospheric pressure chemical ionization ion trap mass spectrometry. Rapid Commun Mass Spectrom 21:3024–3032

Huang W, Li Z, Niu H, Li D, Zhang J (2008) Optimization of operating parameters for supercritical carbon dioxide extraction of lycopene by response surface methodology. J Food Eng 89:298–302

Ibáñez E, Palacios J, Señoráns FJ, Santa-María G, Tabera J, Reglero G (2000) Isolation and separation of tocopherols from olive by-products with supercritical fluids. J Am Oil Chem Soc 77:187–190

Jacques PF, Chylack LT (1991) Epidemiologic evidence of a role for the antioxidant vitamins and carotenoids in cataract prevention. Am J Clin Nutr 53:352S–355S

Jahaniaval F, Kakuda Y, Marcone MF (2000) Fatty acid and triacylglycerol compositions of seed oils of five Amaranthus accessions and their comparison to other oils. J Am Oil Chem Soc J Am Oil Chem Soc. Springer-Verlag

Jaime L, Rodriguez-Meizoso I, Cifuentes A, Santoyo S, Suarez S, Ibanez E, Senorans FJ (2010) Pressurized liquids as an alternative process to antioxidant carotenoids' extraction from *Haematococcus pluvialis* microalgae. Lwt-Food Sci Technol 43:105–112

Jones PJ, Macdougall DE, Ntanios F, Vanstone CA (1997) Dietary phytosterols as cholesterol-lowering agents in humans. Can J Physiol Pharmacol 75:217–227

Kagliwal LD, Patil SC, Pol AS, Singhal RS, Patravale VB (2011) Separation of bioactives from seabuckthorn seeds by supercritical carbon dioxide extraction methodology through solubility parameter approach. Sep Purif Technol 80:533–540

Kassama LS, Shi J, Mittal GS (2008) Optimization of supercritical fluid extraction of lycopene from tomato skin with central composite rotatable design model. Sep Purif Technol 60:278–284

Kawahito Y, Kondo M, Machmudah S, Sibano K, Sasaki M, Goto M (2008) Supercritical CO_2 extraction of biological active compounds from loquat seed. Sep Purif Technol 61:130–135

Kelly GS (1999) Squalene and its potential clinical uses. Altern Med Rev J Clin Ther 4:29–36

Kerrola K (1995) Literature review: isolation of essential oils and flavor compounds by dense carbon dioxide. Food Rev Int 11:547–573

Khor HT, Chieng DY (1997) Lipidaemic effects of tocotrienols, tocopherols and squalene: studies in the hamster. Asia Pac J Clin Nutr 6:36–40

Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker BA, Wang J, Yu B, Zhang J, Bryant SH (2015) PubChem substance and compound databases. Nucleic Acids Res

King JW (2000) Sub- and Supercritical fluid processing of agrimaterials: extraction, fractionation and reaction modes. Supercrit Fluids Fundam Appl

King JW, Dunford NT (2002a) Phytosterol-enriched triglyceride fractions from vegetable oil deodorizer distillates utilizing supercritical fluid fractionation technology. Sep Sci Technol 37:451–462

King JW, Dunford NT (2002b) Phytosterol-enriched triglyceride fractions from vegetable oil deodorizer distillates utilizing supercritical fluid fractionation technology. Sep Sci Technol. Taylor & Francis

King JW, Srinivas K (2009) Multiple unit processing using sub- and supercritical fluids. J Supercrit Fluids 20th Year Anniversary Issue J Supercrit Fluids

Kohlmeier L, Hastings SB (1995) Epidemiologic evidence of a role of carotenoids in cardiovascular disease prevention. Am J Clin Nutr 62:1370S–1376S

Kokolakis AK, Golfinopoulos SK (2013) Microwave-assisted techniques (MATs); a quick way to extract a fragrance: a review. Nat Prod Commun 8:1493–1504

Konwarh R, Pramanik S, Kalita D, Mahanta CL, Karak N (2012) Ultrasonication—a complementary "green chemistry" tool to biocatalysis: a laboratory-scale study of lycopene extraction. Ultrason Sonochem 19:292–299

Koo S, Cha K, Song DG, Chung D, Pan CH (2012) Optimization of pressurized liquid extraction of zeaxanthin from *Chlorella ellipsoidea*. J Appl Phycol J Appl Phycol. Springer Netherlands

Kotnik P, Škerget M, Knez Ž (2007) Supercritical fluid extraction of chamomile flower heads: comparison with conventional extraction, kinetics and scale-up. J Supercrit Fluids 43:192–198

Krichnavaruk S, Shotipruk A, Goto M, Pavasant P (2008) Supercritical carbon dioxide extraction of astaxanthin from *Haematococcus pluvialis* with vegetable oils as co-solvent. Bioresour Technol 99:5556–5560

Kunisaki M, Bursell SE, Umeda F, Nawata H, King GL (1998) Prevention of diabetes-induced abnormal retinal blood flow by treatment with d-α-tocopherol. BioFactors 7:55–67

Kwon KT, Uddin MS, Jung GW, Sim JE, Chun BS (2010) Supercritical carbon dioxide extraction of phenolics and tocopherols enriched oil from wheat bran. Int J Biol Life Sci 6:117–122

Lee J, Ye L, Landen J, Eitenmiller RR (2000) Optimization of an extraction procedure for the quantification of vitamin E in tomato and broccoli using response surface methodology. J Food Compos Anal 13:45–57

Leighton TG (2007) What is ultrasound? Prog Biophys Mol Biol Eff Ultrasound Infrasound Relevant Hum Health

Leitner W, Poliakoff M (2008) Supercritical fluids in green chemistry. Green Chem 10:730–735
Li BB, Smith B, Hossain M (2006) Extraction of phenolics from citrus peels: I. Solvent extraction method. Sep Purif Technol Sep Purif Food Ind
Li TSC, Beveridge THJ, Drover JCG (2007) Phytosterol content of sea buckthorn (*Hippophae rhamnoides L.*) seed oil: Extraction and identification. Food Chem 101:1633–1639
Li Y, Radoiu M, Fabiano-Tixier AS, Chemat F (2013) From laboratory to industry: scale-up, quality, and safety consideration for microwave-assisted extraction. Microwave-Assist Extr Bioact Compd. Springer
Lianfu Z, Zelong L (2008) Optimization and comparison of ultrasound/microwave assisted extraction (UMAE) and ultrasonic assisted extraction (UAE) of lycopene from tomatoes. Ultrason Sonochem 15:731–737
Lim G-B, Lee S-Y, Lee E-K, Haam S-J, Kim W-S (2002) Separation of astaxanthin from red yeast *Phaffia rhodozyma* by supercritical carbon dioxide extraction. Biochem Eng J 11:181–187
Liu J (2005) A combination of mitochondrial nutrients for relieving stress, preventing and improving stress-related disorders. Google Pat
Liu GM, Xu X, Gong Y, He L, Gao YX (2012) Effects of supercritical CO_2 extraction parameters on chemical composition and free radical-scavenging activity of pomegranate (*Punica granatum L.*) seed oil. Food Bioprod Process 90:573–578
London RS, Sundaram G, Murphy L, Goldstein P (1983) The effect of alpha-tocopherol on premenstrual symptomatology: a double-blind study. J Am Coll Nutr 2:115–122
Longo C, Leo L, Leone A (2012) Carotenoids, fatty acid composition and heat stability of supercritical carbon dioxide-extracted-oleoresins. Int J Mol Sci 13:4233–4254
Lopes BLF, Sanchez-Camargo AP, Ferreira ALK, Grimaldi R, Paviani LC, Cabral FA (2012) Selectivity of supercritical carbon dioxide in the fractionation of fish oil with a lower content of EPA plus DHA. J Supercrit Fluids 61:78–85
López M, Arce L, Garrido J, Rios A, Valcarcel M (2004) Selective extraction of astaxanthin from crustaceans by use of supercritical carbon dioxide. Talanta 64:726–731
Lu B, Zhang Y, Wu X, Shi J (2007) Separation and determination of diversiform phytosterols in food materials using supercritical carbon dioxide extraction and ultraperformance liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry. Anal Chim Acta 588:50–63
Lucchesi ME, Chemat F, Smadja J (2004) Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro-distillation. J Chromatogr A 1043:323–327
Lungershausen YK, Abbey M, Nestel PJ, Howe PR (1994) Reduction of blood pressure and plasma triglycerides by omega-3 fatty acids in treated hypertensives. J Hypertens 12:1041–1046
Luque De Castro MD, Priego-Capote F (2010) Soxhlet extraction: past and present panacea. J Chromatogr Extr Tech
Machmudah S, Winardi S, Sasaki M, Goto M, Kusumoto N, Hayakawa K (2012) Lycopene extraction from tomato peel by-product containing tomato seed using supercritical carbon dioxide. J Food Eng 108:290–296
Macias-Sanchez MD, Fernandez-Sevilla JM, Fernandez FGA, Garcia MCC, Grima EM (2010) Supercritical fluid extraction of carotenoids from Scenedesmus almeriensis. Food Chem 123:928–935
Marubayashi S, Dohi K, Ochi K, Kawasaki T (1986) Role of free radicals in ischemic rat liver cell injury: prevention of damage by alpha-tocopherol administration. Surgery 99:184–192
Mason TJ, CHEMAT F, VINATORU M (2011) The extraction of natural products using ultrasound or microwaves. Curr Org Chem 15:237–247
Mayne ST (1996) Beta-carotene, carotenoids, and disease prevention in humans. FASEB J 10:690–701
McClements DJ, Decker EA, Weiss J (2007) Emulsion-based delivery systems for lipophilic bioactive components. J Food Sci 72:R109–R124

Mendes RL, Nobre BP, Cardoso MT, Pereira AP, Palavra ANF (2003) Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. Inorg Chim Acta Protagonists Chem. Frausto Da Silva

Mendiola JA, Herrero M, Castro-Puyana M, Ibáñez E (2013) Supercritical fluid extraction. Nat Prod Extr Principles Appl 21:196

Meydani SN, Wu D, Santos MS, Hayek MG (1995) Antioxidants and immune response in aged persons: overview of present evidence. Am J Clin Nutr 62:1462S–1476S

Mezzomo N, Mileo BR, Friedrich MT, Martinez J, Ferreira SR (2010) Supercritical fluid extraction of peach (*Prunus persica*) almond oil: process yield and extract composition. Bioresour Technol 101:5622–5632

Moghadasian MH, Frohlich JJ (1999) Effects of dietary phytosterols on cholesterol metabolism and atherosclerosis: clinical and experimental evidence. Am J Med 107:588–594

Molero G, MARTINEZ DE LA OSSA (2000) Quality of wheat germ oil extracted by liquid and supercritical carbon dioxide. J Am Oil Chem Soc 77:969–974

Moura PM, Prado GHC, Meireles MAA, Pereira CG (2012) Supercritical fluid extraction from guava (*Psidium guajava*) leaves: Global yield, composition and kinetic data. J Supercrit Fluids 62:116–122

Moyler DA (1993) Extraction of essential oils with carbon dioxide. Flavour Fragrance J 8:235–247

Mustafa A, Turner C (2011) Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review. Anal Chim Acta 703:8–18

Nobre BP, Palavra AF, Pessoa FLP, Mendes RL (2009) Supercritical CO_2 extraction of trans-lycopene from Portuguese tomato industrial waste. Food Chem 116:680–685

Nyam KL, Tan CP, Lai OM, Long K, Man YBC (2010) Optimization of supercritical fluid extraction of phytosterol from roselle seeds with a central composite design model. Food Bioprod Process 88:239–246

Nyam K, Tan C, Lai O, Long K, Che Man Y (2011) Optimization of supercritical CO_2 extraction of phytosterol-enriched oil from Kalahari melon seeds. Food Bioprocess Technol Food Bioprocess Technol. Springer-Verlag

Oddy W, de Klerk N, Kendall G, Mihrshahi S, Peat J (2004) Ratio of omega-6 to omega-3 fatty acids and childhood asthma. J Asthma 41:319–326

Okoh OO, Sadimenko AP, Afolayan AJ (2010) Comparative evaluation of the antibacterial activities of the essential oils of *Rosmarinus officinalis L.* obtained by hydrodistillation and solvent free microwave extraction methods. Food Chem 120:308–312

Ollanketo M, Hartonen K, Riekkola ML, Holm Y, Hiltunen R (2001) Supercritical carbon dioxide extraction of lycopene in tomato skins. Eur Food Res Technol 212:561–565

Olmedilla B, Granado F, Blanco I, Vaquero M (2003) Lutein, but not α-tocopherol, supplementation improves visual function in patients with age-related cataracts: a 2-y double-blind, placebo-controlled pilot study. Nutrition 19:21–24

Ostlund RE (2002) Phytosterols in human nutrition. Annu Rev Nutr. Annual Reviews

Oufnac DS, Xu Z, Sun T, Sabliov C, Prinyawiwatkul W, Godber JS (2007) Extraction of antioxidants from wheat bran using conventional solvent and microwave-assisted methods. Cereal Chem J 84:125–129

Palavra AMF, Coelho JP, Barroso JG, Rauter AP, Fareleira JMNA, Mainar A, Urieta JS, Nobre BP, Gouveia L, Mendes RL, Cabral JMS, Novais JM (2011) Supercritical carbon dioxide extraction of bioactive compounds from microalgae and volatile oils from aromatic plants. In: The journal of supercritical fluids selected papers from the 9th conference on supercritical fluids and their applications, 05–08 Sept 2010, Sorrento (Italy)

Palma M, Barbero GF, Pineiro Z, Liazid A, Barroso CG, Rostagno MA, Prado JM, Meireles MAA (2013) Chapter 2 extraction of natural products: principles and fundamental aspects. Nat Prod Extr Principles Appl. The Royal Society of Chemistry

Pasquet V, Cherouvrier JR, Farhat F, Thiery V, Piot JM, Berard JB, Kaas R, Serive B, Patrice T, Cadoret JP, Picot L (2011) Study on the microalgal pigments extraction process: performance of microwave assisted extraction. Process Biochem 46:59–67

Pereira C, Meireles A (2010) Supercritical fluid extraction of bioactive compounds: fundamentals, applications and economic perspectives. Food Bioprocess Technol Food Bioprocess Technol. Springer-Verlag

Perera CO, Yen GM (2007) Functional properties of carotenoids in human health. Int J Food Prop. Taylor & Francis

Peres VF, Saffi J, Melecchi MI, Abad FC, De Assis Jacques R, Martinez MM, Oliveira EC, Caramao EB (2006) Comparison of soxhlet, ultrasound-assisted and pressurized liquid extraction of terpenes, fatty acids and vitamin E from piper gaudichaudianum Kunth

Perretti G, Troilo A, Bravi E, Marconi O, Galgano F, Fantozzi P (2013) Production of a lycopene-enriched fraction from tomato pomace using supercritical carbon dioxide. J Supercrit Fluids 82:177–182

Pingret D, Fabiano-Tixier AS, Chemat F (2013) Chapter 3 ultrasound-assisted extraction. Nat Prod Extr Principles Appl. The Royal Society of Chemistry

Plaza M, Amigo-Benavent M, del Castillo MD, Ibanez E, Herrero M (2010a) Neoformation of antioxidants in glycation model systems treated under subcritical water extraction conditions. Food Res Int 43:1123–1129

Plaza M, Santoyo S, Jaime L, Garcia-Blairsy Reina G, Herrero M, Senorans FJ, Ibanez E (2010b). Screening for bioactive compounds from algae. J Pharm Biomed Anal Nat Bioact Compd Nutrigenomics

Priego-Capote F, Delgado De La Torre MDP (2013) Chapter 5 accelerated liquid extraction. Nat Prod Extr Principles Appl. The Royal Society of Chemistry

Pronyk C, Mazza G (2009) Design and scale-up of pressurized fluid extractors for food and bioproducts. J Food Eng 95:215–226

Ranasinghe L, Jayawardena B, Abeywickrama K (2002) Fungicidal activity of essential oils of *Cinnamomum zeylanicum* (L.) and *Syzygium aromaticum* (L.) Merr et LM Perry against crown rot and anthracnose pathogens isolated from banana. Lett Appl Microbiol 35:208–211

Rao CV, Newmark HL, Reddy BS (1998) Chemopreventive effect of squalene on colon cancer. Carcinogenesis 19:287–290

Rao AV, Rao LG (2007) Carotenoids and human health. Pharmacol Res Nutr Pharmacol

Reverchon E (1997) Supercritical fluid extraction and fractionation of essential oils and related products. J Supercrit Fluids 10:1–37

Reverchon E, de Marco I (2006) Supercritical fluid extraction and fractionation of natural matter. J Supercrit Fluids 38:146–166

Reverchon E, Senatore F (1992) Isolation of rosemary oil: comparison between hydrodistillation and supercritical CO_2 extraction. Flavour Fragrance J 7:227–230

Riccioni G (2009) Carotenoids and cardiovascular disease. Curr Atherosclerosis Rep 11:434–439

Rodriguez-Meizoso I, Jaime L, Santoyo S, Senorans FJ, Cifuentes A, Ibanez E (2010) Subcritical water extraction and characterization of bioactive compounds from *Haematococcus pluvialis* microalga. J Pharm Biomed Anal Nat Bioact Compd Nutrigenomics

Routray W, Orsat V (2012) Microwave-assisted extraction of flavonoids: a review. Food Bioprocess Technol Food Bioprocess Technol. Springer-Verlag

Rozzi NL, Singh RK, Vierling RA, Watkins BA (2002) Supercritical fluid extraction of lycopene from tomato processing by-products. J Agric Food Chem. American Chemical Society

Rubio-Rodriguez N, Beltran S, Jaime I, de Diego SM, Sanz MT, Carballido JR (2010) Production of omega-3 polyunsaturated fatty acid concentrates: a review. Innovative Food Sci Emerg Technol 11:1–12

Rubio-Rodríguez N, de Diego SM, Beltrán S, Jaime I, Sanz MT, Rovira J (2008) Supercritical fluid extraction of the omega-3 rich oil contained in hake (*Merluccius capensis–Merluccius paradoxus*) by-products: study of the influence of process parameters on the extraction yield and oil quality. J Supercrit Fluids 47:215–226

Ruen-Ngam D, Shotipruk A, Pavasant P, Machmudah S, Goto M (2012) Selective extraction of lutein from alcohol treated *Chlorella vulgaris* by supercritical CO_2. Chem Eng Technol 35:255–260

Sabio E, Lozano M, Montero De Espinosa V, Mendes RL, Pereira AP, Palavra AF, Coelho JA (2003) Lycopene and b-carotene extraction from tomato processing waste using supercritical CO_2. Ind Eng Chem Res. American Chemical Society

Sadraei H, Asghari G, Hajhashemi V, Kolagar A, Ebrahimi M (2001) Spasmolytic activity of essential oil and various extracts of *Ferula gummosa* Boiss. on ileum contractions. Phytomedicine 8:370–376

Sahena F, Zaidul ISM, Jinap S, Karim AA, Abbas KA, Norulaini NAN, Omar AKM (2009a) Application of supercritical CO_2 in lipid extraction—a review. J Food Eng 95:240–253

Sahena F, Zaidul ISM, Jinap S, Karim AA, Abbas KA, Norulaini NAN, Omar AKM (2009b) Application of supercritical CO_2 in lipid extraction: a review. J Food Eng 95:240–253

Sajfrtova M, Lickova I, Wimmerova M, Sovova H, Wimmer Z (2010) Beta-sitosterol: supercritical carbon dioxide extraction from sea buckthorn *(Hippophae rhamnoides L.)* seeds. Int J Mol Sci 11:1842–1850

Sajilata MG, Singhal RS, Kamat MY (2008) The carotenoid pigment zeaxanthin—a review. Compr Rev Food Sci Food Saf 7:29–49

Sanchez-Camargo AP, Meireles MAA, Lopes BLF, Cabral FA (2011) Proximate composition and extraction of carotenoids and lipids from Brazilian redspotted shrimp waste (*Farfantepenaeus paulensis*). J Food Eng 102:87–93

Sanchez-Camargo AP, Meireles MAA, Ferreira ALK, Saito E, Cabral FA (2012) Extraction of omega-3 fatty acids and astaxanthin from Brazilian redspotted shrimp waste using supercritical CO_2+ethanol mixtures. J Supercrit Fluids 61:71–77

Sánchez-González L, Vargas M, González-Martínez C, Chiralt A, Cháfer M (2011) Use of essential oils in bioactive edible coatings: a review. Food Eng Rev 3:1–16

Santoyo S, Plaza M, Jaime L, Ibanez E, Reglero G, Senorans J (2011) Pressurized liquids as an alternative green process to extract antiviral agents from the edible seaweed *Himanthalia elongata*. J Appl Phycol J Appl Phycol. Springer Netherlands

Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, Burton TC, Farber MD, Gragoudas ES, Haller J, Miller DT (1994) Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. JAMA 272:1413–1420

Serra AT, Seabra IJ, Braga MEM, Bronze MR, de Sousa HC, Duarte CMM (2010) Processing cherries (*Prunus avium*) using supercritical fluid technology. Part 1: recovery of extract fractions rich in bioactive compounds. J Supercrit Fluids 55:184–191

Sesso HD, Buring JE, Norkus EP, Gaziano JM (2005) Plasma lycopene, other carotenoids, and retinol and the risk of cardiovascular disease in men. Am J Clin Nutr 81:990–997

Shang YF, Kim SM, Lee WJ, Um BH (2011) Pressurized liquid method for fucoxanthin extraction from *Eisenia bicyclis* (Kjellman) Setchell. J Biosci Bioeng 111:237–241

Shi J, Yi C, Xue SJ, Jiang Y, Ma Y, Li D (2009) Effects of modifiers on the profile of lycopene extracted from tomato skins by supercritical CO_2. J Food Eng 93:431–436

Shi J, Xue SJ, Ma Y, Jiang Y, Ye X, Yu D (2012) Green separation technologies in food processing: supercritical-CO_2 fluid and subcritical water extraction green technologies in food production and processing. In: Boye JI, Arcand Y (eds). Springer US

Sihvonen M, Jarvenpaa E, Hietaniemi V, Huopalahti R (1999) Advances in supercritical carbon dioxide technologies. Trends Food Sci Technol 10:217–222

Silva I (2013) Evaluation of chemotherapeutic potential of natural extracts using 3D models of colon cancer

Silva J, Abebe W, Sousa S, Duarte V, Machado M, Matos F (2003) Analgesic and anti-inflammatory effects of essential oils of *Eucalyptus*. J Ethnopharmacol 89:277–283

Simopoulos AP (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 56:365–379

Simopoulos AP (2008) The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med (Maywood) 233:674–688

Simsek M, Sumnu G, Sahin S (2012) Microwave assisted extraction of phenolic compounds from sour cherry pomace. Sep Sci Technol. Taylor & Francis

Smith T (2000) Squalene: potential chemopreventive agent. Expert Opin Investig Drugs. Expert Opinion

Smith TJ, Yang GY, Seril DN, Liao J, Kim S (1998) Inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis by dietary olive oil and squalene. Carcinogenesis 19:703–706

Snodderly DM (1995) Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. Am J Clin Nutr 62:1448S–1461S

Soria AC, Villamiel M (2010) Effect of ultrasound on the technological properties and bioactivity of food: a review. Trends Food Sci Technol 21:323–331

Sovová H (2012) Modeling the supercritical fluid extraction of essential oils from plant materials. J Chromatogr A 1250:27–33

Stahl W, Sies H (2005) Bioactivity and protective effects of natural carotenoids. Biochim Biophy Acta (BBA)-Mol Basis Dis 1740:101–107

Starmans DAJ, Nijhuis HH (1996) Extraction of secondary metabolites from plant material: a review. Trends Food Sci Technol 7:191–197

Szentmihalyi K, Vinkler P, Lakatos B, Illes V, Then M (2002) Rose hip (*Rosa canina L.*) oil obtained from waste hip seeds by different extraction methods. Bioresour Technol 82:195–201

Tam CU, Yang FQ, Zhang QW, Guan J, Li SP (2007) Optimization and comparison of three methods for extraction of volatile compounds from *Cyperus rotundus* evaluated by gas chromatography–mass spectrometry. J Pharm Biomed Anal 44:444–449

Tang SK, Qin CR, Wang HG, Li SF, Tian SJ (2011) Study on supercritical extraction of lipids and enrichment of DHA from oil-rich microalgae. J Supercrit Fluids 57:44–49

Tao Y, Sun DW (2013) Enhancement of food processes by ultrasound: a review. Crit Rev Food Sci Nutr. Taylor & Francis

Topal U, Sasaki M, Goto M, Hayakawa K (2006) Extraction of lycopene from tomato skin with supercritical carbon dioxide: effect of operating conditions and solubility analysis. J Agric Food Chem. American Chemical Society

Vagi E, Simandi B, Vasarhelyine KP, Daood H, Kery A, Doleschall F, Nagy B (2007) Supercritical carbon dioxide extraction of carotenoids, tocopherols and sitosterols from industrial tomato by-products. J Supercrit Fluids 40:218–226

Vasapollo G, Longo L, Rescio L, Ciurlia L (2004) Innovative supercritical CO_2 extraction of lycopene from tomato in the presence of vegetable oil as co-solvent. J Supercrit Fluids 29:87–96

Vasquez L, Torres CF, Fornari T, Senorans F, Reglero G (2007) Recovery of squalene from vegetable oil sources using countercurrent supercritical carbon dioxide extraction. J Supercrit Fluids 40:59–66

Vicente G, Molina S, Gonzalez-Vallinas M, Garcia-Risco MR, Fornari T, Reglero G, De Molina AR (2013) Supercritical rosemary extracts, their antioxidant activity and effect on hepatic tumor progression. In: The journal of supercritical fluids special issue—10th international symposium on supercritical fluids special issue—10th international symposium on supercritical fluids

Vidovic S, Mujic I, Zekovic Z, Lepojevic Z, Milosevic S, Jokic S (2011) Extraction of fatty acids from boletus edulis by subcritical and supercritical carbon dioxide. J Am Oil Chem Soc J Am Oil Chem Soc. Springer-Verlag

von Schacky C, Angerer P, Kothny W, Theisen K, Mudra H (1999) The effect of dietary omega-3 fatty acids on coronary atherosclerosis. Ann Intern Med 130:554–562

Wang L, Weller CL (2006) Recent advances in extraction of nutraceuticals from plants. Trends Food Sci Technol 17:300–312

Wang L, Weller CL, Schlegel VL, Carr TP, Cuppett SL (2008) Supercritical CO_2 extraction of lipids from grain sorghum dried distillers grains with solubles. Bioresour Technol 99:1373–1382

Wang Y, Sun D, Chen H, Qian L, Xu P (2011) Fatty acid composition and antioxidant activity of tea (*Camellia sinensis L.*) seed oil extracted by optimized supercritical carbon dioxide. Int J Mol Sci 12:7708–7719

Weber A, Catchpole O, Eltringham W (2008) Supercritical fluid assisted, integrated process for the synthesis and separation of different lipid derivatives. J Sep Sci 31:1346–1351

Xiao W, Han L, Shi B (2008) Optimization of microwave: assisted extraction of flavonoid from radix astragali using response surface methodology. Sep Sci Technol. Taylor & Francis

Xiao X-H, Yuan Z-Q, Li G-K (2013) Preparation of phytosterols and phytol from edible marine algae by microwave-assisted extraction and high-speed counter-current chromatography. Sep Purif Technol 104:284–289

Xu LL, Zhan XR, Zeng ZW, Chen R, Li HF, Xie T, Wang SL (2011a) Recent advances on supercritical fluid extraction of essential oils. Afr J Pharm Pharmacol 5:1196–1211

Xu X, Dong J, Mu X, Sun L (2011b) Supercritical CO_2 extraction of oil, carotenoids, squalene and sterols from lotus (*Nelumbo nucifera Gaertn*) bee pollen. Food Bioprod Process 89:47–52

Yamaguchi K, Murakami M, Nakano H, Konosu S, Kokura T, Yamamoto H, Kosaka M, Hata K (1986). Supercritical carbon dioxide extraction of oils from Antarctic krill. J Agric Food Chem. American Chemical Society

Yamashita T, Oda E, Sano T, Yamashita T, Ijiru Y, Giddings J, Yamamoto J (2005) Varying the ratio of dietary n−6/n−3 polyunsaturated fatty acid alters the tendency to thrombosis and progress of atherosclerosis in apoE−/−LDLR−/−double knockout mouse. Thromb Res 116:393–401

Yi C, Shi J, Xue SJ, Jiang Y, Li D (2009) Effects of supercritical fluid extraction parameters on lycopene yield and antioxidant activity. Food Chem 113:1088–1094

Zhang HF, Yang XH, Wang Y (2011) Microwave assisted extraction of secondary metabolites from plants: current status and future directions. Trends Food Sci Technol Spec Sect Recent Dev Food Sci Technol Jpn

Zhao L, Zhao G, Chen F, Wang Z, Wu J, Hu X (2006) Different effects of microwave and ultrasound on the stability of (all-E)-astaxanthin. J Agric Food Chem. American Chemical Society

Zhou DY, Tong LEI, Zhu BW, Wu HT, Qin LEI, Tan HUI, Murata YOSH (2012) Extraction of lipid from abalone (*Haliotis discus hannai Ino*) Gonad by supercritical carbon dioxide and enzyme-assisted organic solvent methods. J Food Process Preserv 36:126–132

Zhu BW, Qin L, Zhou DY, Wu HT, Wu J, Yang JF, Li DM, Dong XP, Murata Y (2010) Extraction of lipid from sea urchin (*Strongylocentrotus nudus*) gonad by enzyme-assisted aqueous and supercritical carbon dioxide methods. Eur Food Res Technol Eur Food Res Technol. Springer-Verlag

Ziegler RG (1989) A review of epidemiologic evidence that carotenoids reduce the risk of cancer. J Nutr 119:116–122

Zigoneanu IG, Williams L, Xu Z, Sabliov CM (2008) Determination of antioxidant components in rice bran oil extracted by microwave-assisted method. Bioresour Technol 99:4910–4918

Chapter 2
Recovery Technologies for Water-Soluble Bioactives: Advances in Membrane-Based Processes

Alfredo Cassano

Abstract In this chapter, an overview of membrane separation systems in the food industry is provided. Basic principles of pressure-driven membrane operations are described, together with some key advantages and shortcomings compared to conventional technologies. Selected applications of membrane unit operations and integrated membrane systems in specific areas of agro-food production (dairy, soybean, fish processing, olive oil) are also reviewed and discussed, highlighting their potential with respect to the separation, concentration and purification of high-added-value compounds, improvement in food quality and the reduction of environmental pollution.

Keywords Food processing · Pressure-driven membrane operations · Microfiltration (MF) · Ultrafiltration (UF) · Nanofiltration (NF) · Reverse osmosis (RO)

Nomenclature

a	Constant ([-])
b	Constant ([-])
c	Constant ([-])
C	Concentration ([mol m^{-3}, mol L^{-1}])
d	Constant ([-])
d_h	Hydraulic diameter ([m])
D	Diffusion coefficient ([m^2 s^{-1}])
J_s	Solute flux ([mol m^{-2} s^{-1}])
J_v	Volume flux ([m s^{-1}])
k	Mass transfer coefficient ([m s^{-1}])
l	Membrane thickness ([m])
L	Length ([m])
P	Permeability ([mol m^{-1} s^{-1} Pa^{-1}])
p	Pressure ([Pa or bar])

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R Rejection ([-])
R Resistance ([m^{-1}])
Re Reynolds number ([-])
Sc Schmidt number ([-])
Sh Sherwood number ([-])
t Time ([s])
u Velocity ([m s^{-1}])
V Volume ([m^3])
VCR Volume concentration ratio ([-])
WCR Weight concentration ratio ([-])
Y Yield ([-])

Greek Letters

δ Boundary layer thickness ([m])
Δ Difference ([-])
μ Viscosity ([Pa s or m^2 s^{-1}])

Abbreviations

BOD Biological oxygen demand
CA Cellulose acetate
COD Chemical oxygen demand
DF Diafiltration
Igs Immunoglobulins
MF Microfiltration
MPCs Milk protein concentrates
MWCO Molecular weight cut-off
NF Nanofiltration
OMW Olive mill wastewater
PA Polyamide
PAN Polyacrylonitrile
PE Polyethylene
PES Polyethersulphone
PP Polypropylene
PS Polysulphone
PTFE Polytetrafluoroethylene
PVDF Polyvinylidene fluoride
RSM Response surface methodology
RO Reverse osmosis
TOC Total organic carbon
TAA Total antioxidant activity
UF Ultrafiltration
WPCs Whey protein concentrates

Subscripts
b Bulk
c Cake
g Membrane wall (gel)
i Inlet
m Membrane
o Outlet
p Permeate
r Retentate
s Solute
v Volume
0 Initial value

2.1 Introduction

The global demand for membrane modules in water treatment and industrial applications was valued at approximately USD 15.6 billion in 2012. With growth in subsequent years estimated at 8 % annually, the market is forecast to increase to USD 21.2 billion in 2016 and to approach USD 25.0 billion in 2018 (Market Report 2013). In the food industry, the current market for membranes constitutes about 20–30 % of the overall manufacturing industry worldwide, with growth of around 7.5 % per year (Daufin et al. 2001).

Membrane separation processes have special importance in food processing and food wastewater treatments. They can contribute to the simplification of traditional processing steps for the manufacture of food ingredients and to the formulation of novel foods with improved sensory and nutritional characteristics.

Membrane processes offer several advantages over conventional separation techniques such as decantation, centrifugation, filtration, solvent extraction and evaporation (thermal/vacuum concentration). One of the most important advantages in the field of food processing is the ability to operate at room temperature, thus preserving heat-sensitive compounds that can be deactivated or denatured at higher temperature (e.g. proteins). In addition, membrane separation does not involve the use of chemical substances or phase changes, so it is characterized by low energy consumption and reduced environmental impact. Another attractive feature of membrane processes is the simplicity of their layout and their modular design that allows for easy scale-up and increased production capacity.

The membrane market today offers a full range of membranes and systems to maximize the economic use of water and the recovery of valuable compounds from food processing streams in order to meet the special needs of both food processors and environmental discharge standards.

In this chapter, the potential for pressure-driven membrane operations in areas of agro-food production is analyzed and discussed. The first part of the chapter will focus on basic principles of the technology, including typical membrane types and modules

used in different membrane-based operations, and on membrane fouling, which represents a key disadvantage of membrane filtration. The second part of the chapter will provide an outlook on specific applications of pressure-driven membrane operations in different areas of agro-food production (dairy, fish, olive oil, and other foods and beverages), highlighting their potential for the recovery of water and high-added-value compounds from the original solutions, and for reducing the environmental burden of food processing wastewater. The integration of different membrane unit operations at both the laboratory and industrial scale is also considered. This appears to be an attractive way to redesign the conventional production cycles of the agro-food industry within the context of a process intensification strategy, enabling dramatic improvements in manufacturing and processing, and reducing the equipment size/production capacity ratio, energy consumption and waste production.

2.2 Basic Principles of Pressure-Driven Membrane Operations

2.2.1 Membrane Types and Modules

In general, a membrane can be defined as a selective barrier separating two adjacent phases that allows the selective transport of particular species between the two phases in a specific or non-specific manner. Synthetic membranes can be produced from organic materials (polymers) as well as inorganic materials (ceramics, zeolites, metals and their alloys). Membrane chemistry determines important properties such as hydrophilicity or hydrophobicity, the presence or absence of ionic charges, chemical and thermal resistance, binding affinity for solutes or particles, and biocompatibility (Strathmann et al. 2006). Polymeric membranes dominate the industrial membrane technology market because they are highly competitive in performance and affordability. The most common polymeric membranes are manufactured from cellulose acetate (CA), polysulphone (PS), polyethersulphone (PES), polytetrafluoroethylene (PTFE), polypropylene (PP), polyethylene (PE), polyvinylidene fluoride (PVDF), polyacrylonitrile (PAN) and polyamide (PA). Many membrane polymers are grafted, custom-modified or produced as copolymers to improve their properties. Indeed, many studies have sought to enhance the performance of hydrophobic membranes (i.e. PVDF membranes) by introducing hydrophilic functional groups such as $–SO_3H$ of –COOH to the surface by chemical modification or by adding water-soluble polymers such as poly(vinylpyrrolidone) or poly(vinyl methyl ether) to the membrane casting solutions (Baker 2000).

Ceramic membranes are manufactured from inorganic materials (e.g. alumina, zirconia, titania and silica). They exhibit high resistance to aggressive media (acids, alkalis, strong solvents) and high mechanical and thermal stability. Although their production costs are higher than those of polymeric membranes, they are ecologically friendly and durable, and have a longer lifetime.

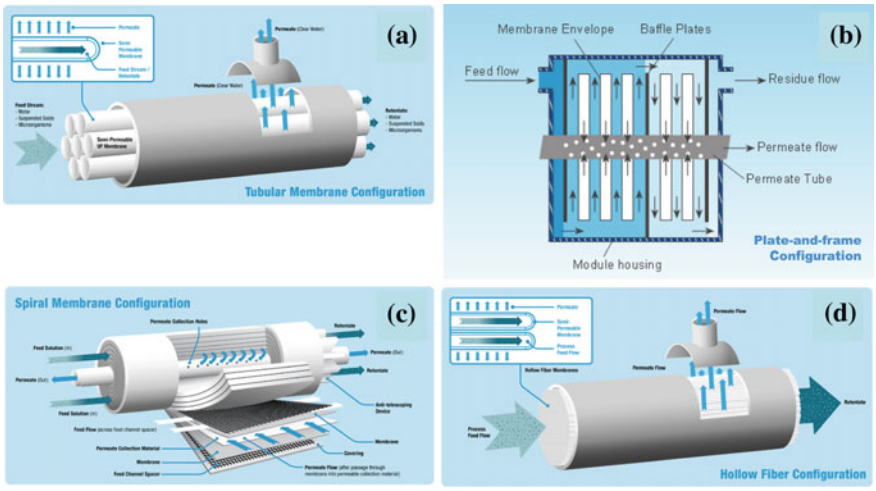

Fig. 2.1 Schematic representation of **a** tubular, **b** plate-and-frame, **c** spiral-wound and **d** hollow fiber membrane module (adapted from Koch Membrane Systems)

On the basis of their structure, synthetic membranes can be classified as porous membranes, homogeneous membranes, solid membranes carrying electrical charges, and liquid or solid films containing selective carriers (Strathmann et al. 2006).

Porous membranes consist of a solid matrix with defined pores having diameters ranging from less than 1 nm to more than 10 μm. They can be classified as follows: macroporous, with average pore diameters larger than 50 nm (i.e. microfiltration and ultrafiltration membranes); mesoporous, with average pore diameters ranging from 2 to 50 nm (i.e. nanofiltration membranes); and microporous, with average pore diameters of between 0.1 and 2 nm. Dense membranes, such as those used in reverse osmosis, have no discrete permanent pores, but separation occurs through fluctuating free volumes.

In addition, the structure of membranes may be symmetric, asymmetric or composite. Symmetric membranes are characterized by identical structure and transport properties over the whole cross-section. They range in thickness from 100 to 200 μm. A decrease in membrane thickness results in an increased permeate flux. Asymmetric membranes consist of a dense 'skin' layer supported by a porous layer providing mechanical strength. The skin layer is characterized by pores with small diameters and thickness of 0.1–1 μm. Separation characteristics are determined by the nature of the membrane material or by the pore size of the skin layer. Composite membranes also have a dense skin layer, but the top and the sub-layer originate from different polymeric materials with different structures. Generally, the top layer is formed by a dense polymer supported by a microporous substrate (Ramakrishna et al. 2011).

Synthetic membranes used at the industrial level are installed in devices called *membrane modules*. On a large industrial scale, membrane modules are generally available in four basic designs: tubular, hollow fiber, spiral-wound and plate-and-frame.

They are quite different in their design, mode of operation, production costs and energy requirements for pumping the feed solution through the module.

Tubular membrane modules (Fig. 2.1a) consist of several polymeric membranes cast on porous paper or plastic tubular inserts. Membrane tubes are assembled in a housing according to a shell-and-tube configuration. The feed solution is pressurized inside the tubes and the permeate is collected in the shell side of the housing. Tube diameters are typically 10–25 mm.

Tubular designs are suitable for feed solutions with high solids content and large suspended particulate, affording easy control of concentration polarization and membrane fouling phenomena. The membrane area, however, is typically small in comparison with that of other configurations.

Plate-and-frame membrane modules (Fig. 2.1b) are mainly used for products with high viscosity and high solids content. Membranes, feed flow spacers and porous permeate support plates are layered between two endplates and placed in a housing. The feed stream is pressurized in the system and passed over the membrane surface. The permeate passes through the membrane into the permeate channel and is then collected into a central permeate collection manifold. Plate-and-frame modules are quite expensive; they are used in a small number of specific applications for separation of components of highly fouling feeds.

Spiral-wound membrane modules (Fig. 2.1c) consist of a sandwich of flat-sheet membranes, spacers and porous permeate flow material wrapped around a central permeate collecting tube. The feed solution is pumped into the spacer channel tangentially to the membrane surface; the permeate passes through the membrane into a porous permeate channel reaching the permeate collecting tube. Due to their compact layout and high membrane surface-to-volume ratio, spiral-wound elements offer cost-effective solutions for treating large feed volumes. However, the treatment of viscous materials or materials with high solids content is difficult, and feed pretreatment is required (Schwinge et al. 2004). Commercial systems are about 1 m in length, with diameters between 10 and 60 cm. Membrane area can range from 3 to 60 m^2.

Hollow fiber membrane modules (Fig. 2.1d) have a design similar to that of tubular systems but with a smaller membrane diameter (the inside diameter of fibers may range from 0.5 to 1.2 mm). These modules offer high packing density and can withstand relatively high pressure. They can also be used for small volume filtration, and the feed can be supplied either inside or outside the fibers. However, the treatment of viscous feeds and solutions containing particulate matter is difficult. Consequently, extensive pretreatment of the solution is needed in order to remove particles, macromolecules or other materials that can precipitate at the membrane surface. Due to the self-supporting nature of the membranes, hollow fiber systems can be backwashed to aid in cleaning; in this case, the permeate flow is reversed to dislodge the fouling material from the membrane surface, allowing easier control of concentration polarization and fouling phenomena.

The selection of a specific membrane system depends on several aspects including cost, packing density, risk of membrane plugging and ease of cleaning. Feed and membrane properties, as well as operating conditions such as applied pressure, tangential flow velocity and temperature, are all parameters affecting

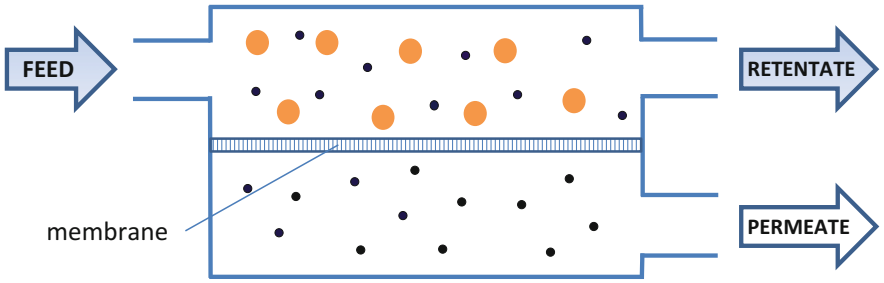

Fig. 2.2 Mechanism of membrane separation

membrane performance. As mentioned above, one of the main limitations of membrane operations lies in membrane fouling, which is associated with the deposition of particles onto the membrane surface and within membrane pores.

2.2.2 Pressure-Driven Membrane Operations

In pressure-driven membrane processes, the membrane acts as a selective barrier through which solvent fluids with permeable solutes are selectively transported under a hydrostatic pressure applied on the feed side. As a result, the feed solution is divided into a permeate fraction containing all components that have permeated the membrane and a retentate fraction containing all compounds rejected by the membrane, within some of the solvent (Fig. 2.2). The separation is based mainly on molecular size, but to a lesser extent on molecular shape, charge and hydrophobicity.

Microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) are typical pressure-driven membrane operation classifications, and represent the key membrane technologies in the food industry.

Microfiltration is used to separate particles with diameters of 0.1–10 μm from a solvent or from low molecular weight compounds. These particles are generally larger than those separated by UF or RO. Consequently, the osmotic pressure for MF is negligible, and hydrostatic pressure differences in MF are relatively small (0.5–4 bar).

Ultrafiltration is a membrane process similar to MF in the mode of separation, based on the use of asymmetric membranes with pore sizes in the skin layer of 2–10 nm. Dissolved molecules or small particles larger than the pore size are retained, though this technique is generally not used for separating particles larger than 0.1 μm in diameter. UF membranes are typically characterized by their molecular weight cut-off (MWCO), defined as the equivalent molecular weight of the smallest species that exhibit 90 % rejection. The MWCO of UF membranes is between 10^3 and 10^6 Da. Hydrostatic pressure of 2–10 bar is typically used.

Nanofiltration membranes are classified between UF and RO membranes in terms of their ability to reject molecular or ionic species, and operate at pressures intermediate between those of UF and RO (generally 3–30 bar). These membranes

are usually characterized by a charged surface with pore diameters of 1–3 nm and MWCO of 200–1000 Da; therefore, components of a fluid are fractionated primarily according to their molecular size and charge. Typically, small uncharged organic molecules and inorganic salts are rejected, especially if multivalent ions are involved. This may lead to significant osmotic pressure across the membrane, which reduces the driving force for the mass transport. Therefore, the use of membranes with high affinity for the solvent ensures high solvent flux and high solute rejection.

Reverse osmosis membranes are typically used to separate low molecular weight compounds from water. Particle sizes for RO applications range from 0.1 to 1 nm, and solutes with molecular weight of approximately 150 Da or less are separated. Since the osmotic pressure of a solution containing low molecular weight solutes can be rather high, hydrostatic pressure on the order of 10–100 bar is applied as driving force in RO to obtain significant transmembrane flux.

The separation capacity of pressure-driven membrane operations is illustrated in Fig. 2.3, which shows that the dimensions of the components involved in these separations range from less than 10^{-3} μm to over 1 μm.

The separation characteristics of membranes can be expressed in terms of membrane rejection (or retention) according to the following equation:

$$R = \left(1 - \frac{C_p}{C_r}\right) \cdot 100 \tag{2.1}$$

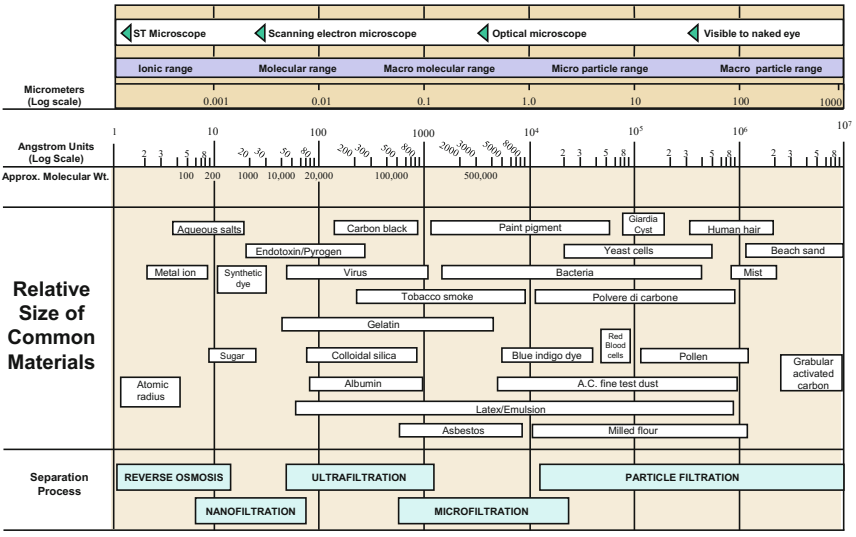

Fig. 2.3 Separation capability of pressure-driven membrane operations (adapted from Osmonics, Inc.)

where R is the membrane rejection for a given component in defined conditions of hydrostatic pressure and feed solution concentration, while C_p and C_r are the concentrations of the component in the permeate and retentate streams, respectively. However, the concentration in these streams depends not only on membrane rejection, but also on the volume concentration ratio (VCR), which for a batch process is defined as:

$$VCR = \frac{V_o}{V_r(t)} = \frac{V_o}{V_o - V_p(t)} \tag{2.2}$$

where V_o is the initial feed volume (L) of the feed, $V_r(t)$ is the final volume (L) of the retentate at particular time (t), and $V_p(t)$ is the volume collected on the permeate side at a particular time (Singh et al. 2013).

Another important parameter is the yield (Y) of a component, which is the fraction of a specific component in the original feed recovered in the final retentate. It is given by:

$$Y = \frac{C_r V_r}{C_0 V_0} \tag{2.3}$$

where C_0 is the initial feed concentration.

For a batch process, the yield of a particular component is an exponential function of the decreasing volume of the feed in the system, according to the following equation:

$$Y = (VCR)^{R-1} \tag{2.4}$$

In a continuous single- or multistage process, the steady state is achieved at higher levels of concentration. For such a process, the yield is given by:

$$Y = \frac{1}{VCR - R(VCR - 1)} \tag{2.5}$$

and the concentration of a component in the final retentate can be calculated from the following equation:

$$C_r = C_0 Y(VCR) \tag{2.6}$$

In MF and UF processes, the retention is affected mainly by the pore size due to the sieving effect; in NF and RO, the membrane retention is affected mainly by electrostatic forces, as well as by other interactions between membrane and solutes. Therefore, membrane retention is also affected by factors such as polarity and solute–membrane interactions, and the MWCO provides only an initial prediction of the membrane's capacity for removing specific compounds (Guizard and Amblard 2009).

The permeate flux through the filter medium is affected by the applied pressure difference across the membrane (between the filtrate and permeate sides of the membrane), the resistance of the membrane and of the cake layer and the viscosity of the fluid being filtered.

The volumetric flux (J_v) is expressed using Darcy's law and a resistance-in-series model:

$$J_v = \frac{\Delta p}{\mu(R_m + R_c)} \tag{2.7}$$

where Δp is the transmembrane pressure (Pa), μ is the permeate viscosity (Pas), R_m is the membrane resistance (m^{-1}) and R_c is the cake resistance (m^{-1}).

Δp is generally calculated as follows:

$$\Delta p = \frac{p_i + p_o}{2} - p_p \tag{2.8}$$

where p_i is the pressure at the inlet of the membrane module, p_o is the pressure at the outlet of the membrane module and p_p is the permeate pressure.

Pressure-driven membrane operations can be performed in either dead-end or tangential-flow configurations. In the dead-end mode, the feed is forced perpendicularly to the membrane surface, and the retained particles tend to form a cake layer on the membrane surface, the thickness of which increases with the filtration time. Therefore, the permeation rate decreases with increasing cake layer thickness. In the tangential-flow mode, the feed solution flows tangentially to the membrane surface and the permeate passes through the membrane due to the imposed transmembrane pressure difference. Unlike in dead-end filtration, the cake layer formed by rejected particles on the membrane surface does not build up indefinitely, but quickly reaches a steady state. The cake layer remains relatively thin due to the high shear exerted by the solution flowing tangential to the membrane surface, clearing the deposited particles towards the retentate exit.

2.2.3 Concentration Polarization and Membrane Fouling

In membrane separation processes, the separation takes place in a very small region close to the membrane, called the *boundary layer*, as well as over the membrane itself (Fig. 2.4). This results in a higher local concentration of the rejected species at the membrane surface compared to the bulk, enhanced by the depletion of the permeating components in the boundary layer adjacent to the membrane surface. This local solute concentration build-up is known as *concentration polarization*, and is chiefly responsible for the marked deviation in flux from that of pure water.

In RO, small solutes are separated from a solvent through a dense membrane. The feed solutions are characterized by high osmotic pressures, and concentration

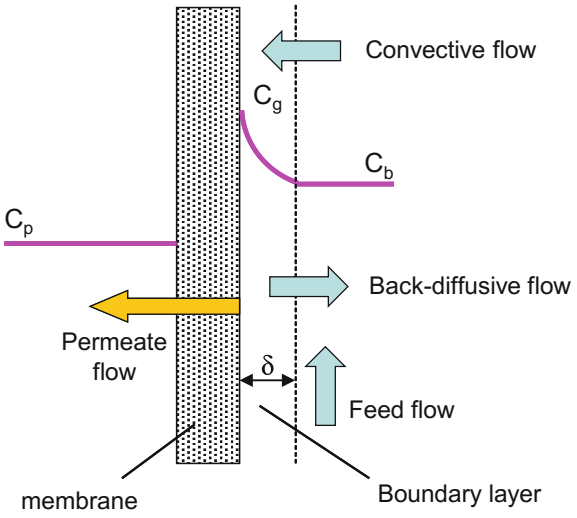

Fig. 2.4 Concentration profile during membrane processing of partially or completely rejected solutes (c_p = permeate concentration; c_g = membrane wall (gel) concentration; c_b = bulk concentration; δ = boundary layer thickness)

polarization leads to an increase in osmotic pressure that is proportional to the solute concentration at the membrane surface. As a consequence, a decrease in permeate flux is observed at a constant applied Δp in both batch and continuous processes. In MF and UF, only macromolecules are retained by the membrane, and the osmotic pressure difference between the feed and the permeate solution is relatively low. Consequently, in these processes, the pressure difference required for operation is also low, but the diffusion of macromolecules from the membrane surface back into the bulk solution is slow. Under these conditions, the retained components can precipitate on the membrane surface, leading to the formation of a solid layer, which affects membrane performance by reducing the transmembrane flux and modifying the selectivity of the membrane.

According to another view, the flux decay can be explained by assuming that the solute accumulated on the membrane surface creates hydrodynamic resistance to the solvent flow in addition to membrane resistance. A dense particle layer is formed by small particles, while large macromolecules form a 'gel' layer. The formation of this gel layer on the membrane surface affects membrane separation characteristics by changing the rejection selectivity towards low molecular weight compounds. In addition, concentration polarization is responsible for the existence of a limiting flux when a feed solution is filtered through a semipermeable membrane. At low pressures, the relationship between permeate flux and applied pressure is linear, and is similar to that of pure solvents. As the pressure is increased, the flux deviates from linear flux–pressure behaviour to a point at which it becomes independent of pressure. The gel layer is assumed to be dynamic; therefore, increasing the tangential flow velocity or decreasing operating pressure or feed solute concentration can cause the system to revert back to a higher and more pressure-responsive permeation rate.

According to the *film model* theory, mass transport resistance is due to a film of liquid near the membrane; a steady state is established by the convective transport of solutes to the membrane surface and the diffusive flux of retained material back into the bulk solution. This is expressed by the following balance equation (Bhattacharya and Hwang, 1997):

$$J_s = J_v C_b + D\frac{dC}{dx} = 0 \tag{2.9}$$

where J_s is the solute flux, J_v is the membrane volume flux, C_b the bulk concentration of the rejected solute, D the diffusion coefficient, and dC/dx the concentration gradient of the solute in the boundary layer (see Fig. 2.4).

Integration over the boundary layer of thickness δ gives:

$$J_v = \frac{D}{\delta} ln \frac{(C_m - C_p)}{(C_b - C_p)} \tag{2.10}$$

where C_m is the solute concentration at the membrane surface, C_p is the solute concentration in the permeate, and C_b, the solute concentration in the bulk.

Introducing the solute mass transfer coefficient $k = D/\delta$ into Eq. (2.10) leads to:

$$J_v = k\, ln \frac{(C_m - C_p)}{(C_b - C_p)} \tag{2.11}$$

which is the general form of the equation for any component.

When the solute concentration at the membrane surface reaches a value at which a 'gel' is formed ($C_m = C_g$), the equation is reduced to the form:

$$J_v = k\, ln \left(\frac{C_g}{C_b}\right) \tag{2.12}$$

The term C_g/C_b refers to the membrane polarization ratio.

Empirical equations correlate the mass transfer coefficient to the channel geometry and flow regimes. This correlation can be expressed in a general form as (Sherwood et al. 1975):

$$Sh = aRe^b Sc^c \left(\frac{d_h}{L}\right) d \tag{2.13}$$

where *Sh, Re and Sc* are the Sherwood, Reynolds and Schmidt numbers, respectively; L is the length of the feed flow channel (or tube); d_h is the hydraulic diameter of the feed channel; and a, b, c and d are characteristic constants for different geometries to be experimentally determined.

The Sherwood number is given by:

$$Sh = k\frac{d_h}{D} \tag{2.14}$$

The Schmidt number is given by:

$$Sc = \frac{\mu}{D} \tag{2.15}$$

where μ is the viscosity.

The Reynolds number in a channel (or in a tube) is given by:

$$Re = \frac{d_h u}{\mu} \tag{2.16}$$

where u is the fluid velocity. In general, Re values less than 1800 are considered to indicate laminar flow and Re greater than 4000 indicates turbulent flow.

Methods for reducing concentration polarization include (i) reduction of the applied pressure; (ii) reduction of solids concentration in the feed solution; (iii) hydrodynamic modifications and the use of turbulent promoters to improve mass transfer; (iv) the use of rotating cylinders or vibratory shear-enhanced units; (v) chemical modification of the membrane surface; and (vi) application of an external body force such as an electric field to influence the charge on macromolecules, the use of ultrasound to induce cavitation at the surface, or the injection of inert gas to induce turbulence.

Membrane fouling is a major problem in pressure-driven membrane operations. It can be considered a long-term flux decline caused by the interaction of chemical species and their deposition on the membrane surface or within the membrane pores. This phenomenon can be induced by several mechanisms, including increased interfacial concentration due to concentration polarization, the adsorption of solutes within the pores, partial or complete pore blocking, the formation of a cake from excluded solutes, and the precipitation or gelation of inorganic and organic particulate at the membrane surface. The general effect is an overall increase in resistance to mass transport, reduction in the membrane surface and change in separation properties of the membrane. Indeed, the presence of foulants at the membrane surface or within the pores affects the passage of solutes through the membrane due to the lower porosity and the difference in charge properties of the fouled membrane in comparison with the clean membrane (Schäfer et al. 2000).

The degree of membrane fouling determines the frequency of cleaning required and the lifetime of the membrane; therefore, it has a significant effect on the cost, design and operation of membrane plants (Boerlage et al. 2004).

Different substances can contribute to fouling phenomena, including organic materials (e.g. proteins, polysaccharides and humic acids), colloidal or particulate matter, inorganic compounds (poorly soluble salts and metal hydroxides), chemical reactants and biological components (microorganisms, products of their metabolism, and cell debris).

In addition, the fouling propensity of a membrane is strongly influenced by the membrane material: for example, proteins may bind to the membrane by hydrophobic effects, hydrogen bonding or electrostatic interactions, depending on the membrane material and protein type. Some functional groups of fat substances are similar to those of PVDF and PS membranes, resulting in considerable fouling phenomena.

The ionic strength, pH and electric charge of the feed material are also significant, since they affect the charge of both membrane surface and particles, the conformation and stability of molecules, and the effective size of the cake layer (Vyas et al. 2000).

Several factors can be considered for controlling and minimizing fouling phenomena, including the selection of appropriate feed-stream pretreatment methods, membrane materials and module configurations, modification of membrane properties, and optimization of cleaning procedures.

Feed-stream pretreatment methods, including adsorption onto active carbon, pH adjustment, heat treatment, the addition of complexing agents, and chlorination, aim to remove or to stabilize foulants by changing the properties of the feed solution.

As reported above, the use of tubular and plate-and-frame designs is preferred for feed solutions containing high solids content and large suspended particulate matter.

The use of turbulence promoters, ultrasonic vibrations, or rotating modules aimed at increasing the convective transport of solutes back to the bulk solution is also effective for reducing membrane fouling.

Operating parameters such as flow rate, transmembrane pressure, temperature and feed concentration have great influence on membrane fouling. Generally, an increase in temperature results in a reduction in viscosity and an increase in the diffusion coefficients of molecules, resulting in higher permeation fluxes, although protein denaturation may be a limiting factor.

Higher rates of tangential flow tend to reduce the aggregation of the feed solids in the gel layer, increasing their diffusion back towards the bulk. This leads to an overall reduction in the effect of concentration polarization. However, in some cases the increase in tangential flow velocity effects the removal of larger particles from the membrane surface, but stratification of smaller particles on the membrane surface, with consequent pore plugging.

Generally, an increase in the feed concentration results in a faster decline in the permeate flux with time, due to higher fouling of the membrane in the presence of higher concentrations of foulants.

As reported previously, when a feed solution is treated by MF or UF, a typical flux plateau is observed where an increase in Δp yields no flux increase; the permeate flux approached at a specific Δp limit value is defined as *limiting flux*. This phenomenon is due to a combination of concentration polarization and membrane fouling (Wu et al. 1999). Below a certain flux, known as the *critical flux*, the relationship between the applied pressure and flux may be linear. Therefore, operating below the critical flux can afford greater control of the fouling phenomenon.

Procedures for cleaning to reduce membrane fouling are based on hydraulic, mechanical, electrical and chemical methodologies. The selection of a system depends on the module configuration, the chemical resistance of the membrane and the type of fouling.

Hydraulic cleaning is typically achieved by in situ methods such as back-flushing and back-pulsing. Back-flushing consists in periodically reversing the transmembrane flow to remove foulants from the membrane surface and pores. With this approach, the direction of the permeate flow is reversed at regular intervals (i.e. 5–30 s once every 30 min to several hours). The back-flushing medium can be the permeate, another liquid or a gas. The procedure is carried out with tubular and hollow fiber membranes, which can withstand the reversal in pressure difference.

In back-pulsing, high speeds and pressure fluctuations are employed to dislodge particles accumulated on the membrane surface. Typically, high-pressure pulses (up to 10 bar) are applied for very short intervals (typically <1 s) (Sondhi and Bhave 2001).

The injection of gas bubbles at high velocities through tubular membrane systems is a mechanical method that can be used to create turbulence at the membrane surface in order to dislodge fouling materials. The use of electrical pulses to produce a movement of charged species away from the surface can be also applied for metallic membranes.

Chemical cleaning is generally realized by cleaning-in-place (CIP) methodologies in order to partially or completely restore the permeate flux. The composition of the cleaning solution is strongly dependent on the nature of fouling agents and the properties of the membrane. Alkaline solutions such as NaOH and KOH up to pH 12 are used to solubilize and break down organics and proteins. Acid cleaning solutions at pH 1.5–2.8 are mainly used to remove inorganic foulants.

Cleaning solutions should be pumped through the system at high velocity and low operating pressure in order to avoid the penetration of foulants into membrane pores. Most chemical cleaning solutions complete their action in 30–60 min; enzymatic cleaning agents may require longer times. The temperature of cleaning solutions should be as high as possible, complying with the temperature limitation of the membrane module.

2.3 Milk and Dairy Industry

The food sector of greatest significance for pressure-driven membrane operations is the dairy industry. Most relevant applications cover the treatment of milk, whey and waste streams, and they are well established at the industrial level. Membrane processes are used in the standardization of milk, whey and milk protein concentrations, fractionation of protein hydrolysates, removal of bacteria and fat from milk, concentration of milk for cheese production, demineralization of whey and cheese brine treatments. For some applications, the integration of pressure-driven membrane processes has been integrated into existing installations, resulting in particular benefits in terms of improved quality, development of new products such as functional and health-promoting foods, enhanced process efficiency and better control of the environmental impact (Rosenberg 1995).

2.3.1 Milk Processing

The protein and total solids content of milk can be standardized using UF membranes to overcome variations in composition. These membranes retain proteins, fats, and insoluble and bound salts, allowing the permeation of water, lactose and soluble salts.

The combination of MF and UF membranes is of interest for the fractionation of caseins. The use of MF membranes with a pore size of 0.2 μm enables the production of a permeate fraction containing mostly whey proteins and a retentate enriched in native caseins that can be used for cheese making. The MF permeate can be further treated by UF to produce high-quality whey protein concentrates (WPCs).

Karasau et al. (2010) compared the performance of ceramic and polymeric membranes in the treatment of skim milk for the manufacture of casein; PVDF and ceramic membranes with a pore size of 0.2 μm provided comparable results when operated at comparable values of applied pressure, tangential flow velocity and temperature.

Milk protein concentrates (MPCs) containing 50–85 % proteins can be obtained through a combination of MF and UF processes with diafiltration (DF) and an appropriate adjustment of pH and temperature. These concentrates find application in the manufacture of cheese products, dietary supplements, cultured products and dairy-based beverages.

The Tetra Pak UF technology is an elegant application of membrane technology in semi-hard cheese production, with significant advantages, especially in terms of higher product yield, when compared with traditional methods. In this approach, which is used to produce fresh white and Mediterranean cheese varieties such as feta and queso fresco, the cheese is packed as a liquid and is coagulated inside the final packaging. The process line for semi-automatic production of microbially acidified feta cheese, the Tetra Tebel Casofill® BAF (bacteriologically acidified feta), consists of a UF step in which milk is concentrated about 5.5 times to a final total solids content of 36–39 %. The UF retentate is then pasteurized and homogenized at 75–80 °C. After cooling, it is pumped to the fermentation tank, and finally is subjected to the filling step (Kiers 2001; Tamime et al. 2006).

2.3.2 Whey Processing

Whey is a liquid co-product of the cheese and casein industries. It has a low solids content (up to 5–6 %), and as a waste, it has high biological oxygen demand (BOD = 30–50 g/L for 1000 L of whey), making whey disposal difficult, costly and environmentally detrimental. Whey solids constitute mainly lactose ($\sim$50 g/L), whey proteins ($\sim$6 g/L) such as α-lactalbumin, β-lactoglobulin, bovine serum

albumin, lactoferrin and immunoglobulins, and minerals (~6 g/L) and fat (0.3 g/L).

Whey protein concentrates (WPCs) can be produced using UF membranes with MWCO of ~10 kDa. These membranes allow the separation of lactose and minerals from the proteins, producing a retentate stream, which can be further processed by evaporation and spray drying. The protein content of the final product may be controlled by the degree of concentration during UF. WPCs with protein content of 35–60 % can be obtained at a VCR ranging from 4.5 to 20, respectively.

The lactose and mineral content in WPC can be further reduced using a DF step in which deionized water is continually added to the retentate while lactose and minerals are continuously recovered in the permeate stream. Selecting a proper combination of UF and DF can lead to an improvement in the purity of WPCs of up to 75–80 %. WPCs are important sources of protein for a large variety of food products, including beverages, processed meats, baby foods, yogurt and cottage cheese (Zidney 1998).

Yorgun et al. (2008) investigated the recovery of proteins from white and curd cheese whey using spiral-wound UF, NF and RO membranes. Among the studied membranes, the highest protein recovery and greatest reduction in chemical oxygen demand (COD) were obtained using a PS NF membrane module with an MWCO of 200 Da. The module exhibited an initial permeate flux of 24 L/m^2/h when cheese whey was treated at a Δp of 8 bar and a recirculation flow rate of 2500 L/h. A permeate with a COD of 2787 mg/L (COD removal 97.4 %) was produced, while the protein rejection was 88 %. Further COD removal from the NF permeate required an additional RO stage as a polishing step. This combination allowed the recovery of protein in the NF retentate (first step), while lactose was recovered in the RO retentate (second step), with simultaneous production of clean RO-water as the last permeate.

PVDF UF membranes with MWCO of 6–8 kDa effected protein rejection of between 92 and 98 % in the treatment of milk and whey, with an acceptable permeate flux (30 L/m^2/h) at low pressures (3 bar). A temperature of about 50 °C was considered a limit value for achieving good performance, avoiding denaturation of proteins and damage to membrane materials. The use of PA NF membranes (MWCO 400 Da) enabled the recovery of lactose from milk and whey UF permeates. Through the selection of proper operating conditions (i.e. VCR of 5, Δp of 20 bar and 30 °C), a concentrated solution containing 20–25 % lactose was obtained with a lactose yield higher than 90 %. This solution could be reused in the confectionery industry, while the permeate stream depleted from lactose could be reused for cleaning or irrigation, or as a last resort, discharged as sewage (Atra et al. 2005), with no adverse environmental impact.

MF and UF processes can also be used to isolate immunoglobulins (Igs) from colostrum whey. The MF of colostrum with membranes of 0.1 μm pore size produces a clear permeate, free from fat globules and casein micelles, containing 80 % of the initial Igs. The MF permeate, or serocolostrum, is then concentrated by UF membranes with MWCO of 100 kDa (Piot et al. 2004).

Korhonen et al. (1998) investigated the use of MF, UF and RO membranes in combination with cation-exchange resins for the concentration of Igs. The Ig level of the final freeze-dried concentrates varied from 45 to 75 %.

NF membranes can be used to demineralize whey before evaporation and drying. These membranes retain proteins and multivalent ions (i.e. Ca^{2+}), removing monovalent ions such as Cl^-, Na^+ and K^+. When sweet whey is treated (pH 6.0–6.6) using negatively charged membranes, the retention of proteins (negatively charged at neutral pH) and polyvalent anions results in the accumulation of negative charges in the retentate stream. This leads to increased transmission of Cl^- and OH^- and to partial transmission of Na^+ and K^+ in order to maintain the electroneutrality in the permeate fraction. In the case of acid whey (pH 4.5–5), the membrane is positively charged, and the retention of proteins (positively charged at acidic pH) and polyvalent cations results in increased transmission of Na^+, K^+ and H^+, and partial removal of Cl^- (Gernigon et al. 2011). Desalination of up to 40 % can be achieved through a combination of NF and DF.

Lactic acid is one of the major food preservatives used in the food industry. Conventional fermentative processes for producing lactic acid are characterized by high costs due to the separations steps needed to meet the quality requirements of food grade. NF and RO processes are useful approaches for the removal of lactic acid from fermentation broths in the cell separation step. The transport of lactic acid through NF membranes (AFC80, PCI Membrane and DK2540C, Filtration Engineering) in the treatment of whey ultrafiltrate fermentation broths was investigated by Gonzáles et al. (2008). The rejection selectivity of both membranes towards lactate increased by increasing the pH, due to the electrostatic repulsion between the ions and the membrane surface: as the pH increased from 2.7 to 6, the lactate rejection increased from 45 to 82 % for the AFC80 membrane and from 10 to 91 % for the DK2540C membrane.

2.4 Fish-Processing Wastewater Treatment

Fish industry wastewater contains potentially valuable molecules for the food sector, such as proteins, flavors and aroma compounds, and the recovery of these materials can lead to significant productivity gains (Massé et al. 2008).

Afonso and Bórquez (2002) evaluated the performance of an integrated process based on a combination of MF and UF membranes for the recovery of proteins from the effluents of a fishmeal plant. The MF pretreatment drastically reduced the oil, grease and suspended matter content in the original effluent. A UF ceramic membrane (Carbosep M2, tubular, 15 kDa MWCO) reduced the organic load of the MF permeate and allowed the recovery of valuable raw materials, including proteins. Protein rejection ranged from 49 to 62 %, depending on the operating conditions. The highest protein rejection was achieved at a Δp of 4 bar and a crossflow velocity of 4 m/s. A conceptual process for the treatment of 10 m^3/h of fishmeal effluent was designed. The plant generated 1 m^3/h of concentrate containing 170 g/L of solids

and 112 g/L of proteins. An economic assessment of the integrated MF-UF process for production of 544 tons/year of fishmeal (66 % protein content) yielded a net present worth of US $160,000, a 17 % rate of return, and an 8-year return on investment, indicating the feasibility of the process for protein recovery and pollution reduction (Afonso et al. 2004).

Mameri et al. (1996) also investigated the use of UF for the recovery and concentration of proteins from fishery washing wastewater. A tubular PS membrane with MWCO of 20 kDa and a multichannel ceramic membrane with 0.1-μm pore size produced similar apparent rejection (70–80 %) of proteins, despite their different pore diameters. The UF process allowed an increase in the protein concentration in the feed solution from 5 to 35 g/dm^3, while simultaneously reducing the biochemical oxygen demand (BOD) by 80 %.

2.5 Recovery of Bioactive Compounds from Vegetable Extracts

The last few decades have seen rapidly growing interest in the utilization of phytochemicals in functional foods, given their beneficial effect on human health, lack of toxicity and low cost. Indeed, epidemiological studies have shown a significant positive association between the intake of fruits and vegetables and a reduced risk of chronic diseases such as cancer, cardiovascular disease, diabetes, osteoporosis, Alzheimer's disease and immune disorders (Kaur and Kapoor 2001).

Conventional extraction techniques for the purification of natural products include solvent extraction, ultrasound-assisted extraction, pressurized liquid extraction, enzyme-aided extraction, supercritical fluid extraction, resin-based extraction and alkaline extraction. These methods have some inherent drawbacks, including the degradation of the target compounds due to high temperatures and long extraction times (as in solvent extractions) and health-related risks.

With a number of advantages including high separation efficiency, low energy requirements, mild operating conditions, no additives, simple equipment and easy scale-up, membrane-based technologies can provide interesting alternatives to conventional systems (Li and Chase 2010).

The purification and concentration of phenolic compounds from aqueous vegetable extracts using membrane operations or integrated membrane systems is a topic that is attracting increasing attention. Prodanov et al. (2008) employed UF membranes with MWCO of 10, 30 and 50 kDa for the separation of phenolics in almond skin extracts according to their molecular weight. Low molecular weight phenolic compounds were recovered in the UF permeate, while proanthocyanidin oligomers were obtained from the retentate.

The use of a PES UF membrane with an MWCO of 30 kDa allowed concentration of R-phycoerythrin from a crude extract of the macro-algae *Grateloupia turuturu* without denaturation or accumulation of undesirable molecules. A VCR of

5 was effective for R-phycoerythrin concentration and pre-purification: 100 % of R-phycoerythrin was recovered, while 32.9 % of other proteins and 64.6 % of sugars passed through the membrane (Denis et al. 2009).

UF membranes also proved successful for concentration of phenolic compounds extracted from grape seeds (Nawaz et al. 2006). The maximum amount of polyphenols (about 11 % of the total weight of the seeds) was recovered by combining a solid-to-liquid ratio of 0.2 g/mL with a 50/50 mixture of water and ethanol as extracting liquid, a two-stage extraction, and a UF membrane with a pore size of 0.22 μm. The procedure provided high extraction rates and extraction selectivity, short extraction times and significant labor savings.

The aqueous extraction of *Castanea sativa* leaves combined with a UF process for the concentration and selective recovery of phenolic compounds was investigated by Diaz-Reinoso et al. (2011). A combination of two membranes of 5 and 10 kDa was proposed for producing a retentate with 40 % active compounds. The use of membranes increased the phenolic content by 18 %, whereas the application of an additional precipitation with ethanol resulted in an increase of 36 %. Diaz-Reinoso et al. (2009) also investigated the use of commercial UF and NF membranes for processing aqueous extracts from grape pomace in order to obtain fractions enriched in antioxidant compounds. All selected membranes with MWCO between 250 and 1000 Da exhibited similar rejection of phenols and sugars, and were suitable for concentration purposes.

Reversible and irreversible fouling phenomena contribute to overall deterioration in the performance of UF membranes in the treatment of aqueous solutions containing polyphenolic compounds. Adsorption onto membrane surfaces and pore walls followed by either pore blocking or narrowing appear to be the main fouling mechanisms when PES membranes are used. In addition, membranes with larger pore sizes (MWCO 100 kDa) were found to exhibit less fouling than membranes with smaller pore sizes (MWCO 10 kDa) (Susanto et al. 2009). Filtration studies of phenolic compounds (such as 2-nitrophenol and 2-clorophenol) through thin-film composite polyamide NF and RO membranes suggested the formation of a fouling monolayer on the membrane surface. Such fouling led to increased hydrophobicity and diminished roughness of the membrane surface. As expected, solute rejection was higher for the RO membrane than the NF membrane. (Sotto et al. 2013).

Mello et al. (2010) evaluated the performance of an NF membrane composed of PA and PS (NF90, GE Osmonics, Minnetonka, MN, USA) for the concentration of flavonoids and phenolic compounds from aqueous and ethanolic propolis extracts. No loss of compounds was detected in the permeate solution produced from the aqueous extract (the NF membrane retained around 94 % of the phenolic compounds and 99 % of the flavonoids). As a consequence of the higher amount of compounds extracted by alcohol, lower retention of phenolic compounds was measured when the ethanolic extract was nanofiltered.

Polyimide NF membranes resistant to organic solvents (StarMem 122 and DuraMem 200, Evonik Industries, Essen, Germany) were also shown to be very efficient for the concentration of phenolic substances from ethanol–water propolis extracts (Tylkowski et al. 2010). DuraMem 200 with an MWCO of 200 Da

exhibited rejection higher than 95 % for all investigated compounds, including flavones, flavonols, flavanones, dihydroflavonols and other phenolics. At applied pressure of 30 and 50 bar, stable permeate fluxes of 2.66 and 4.05 L/m^2/h were measured. The performance of the membranes was also evaluated for concentration of biologically active compounds extracted with ethanol from *Sideritis scardica* Griseb, a plant of the Balkan Peninsula recognized for its high antioxidant activity due to the presence of flavonoids and polyphenols (Tylkowski et al. 2011). Experimental results indicated that membrane rejection was strongly related to the MWCO of the membrane, and that separation of flavonoids from low molecular weight polyphenols occurred at MWCO higher than 400 Da.

The concentration of phenolic compounds from aqueous mate (*Ilex paraguariensis* St. Hil.) extracts with a spiral-wound NF membrane (HL2521TF, Osmonics, Minnetonka, MN, USA) of 150–300 Da was investigated by Aguiar Prudêncio et al. (2012). Chlorogenic acid and epigallocatechin gallate were the main antioxidant compounds detected in concentrates obtained at VCR 4 and 6. The concentration of phenolic compounds and antioxidant activity increased by increasing the VCR (Negrão Murakami et al. 2011).

The recovery of phenolic compounds from citrus by-products by membrane-based operations has been also investigated. Bergamot is the common name of a natural citrus fruit (*Citrus bergamia* Risso) produced in southern Italy. The essential oil, which is obtained from the peel by wash-scraping the fruit, is widely used in the cosmetic industry despite the presence of synthetic surrogates on the market. However, the juice has not found a real use in the food industry due to its bitter taste, even though it contains natural phenols with marked anticholesteremic activity (Di Donna et al. 2009). Tubular ceramic NF membranes with MWCO of 450–1000 Da were evaluated for their selectivity towards sugars, organic acid and flavonoids in the treatment of ultrafiltered bergamot juice. Suspended solids were removed in the preliminary UF step, reducing fouling phenomena in the subsequent NF step. The best separation of polyphenols from sugars occurred with the NF 450-Da membrane, which exhibited the highest rejection selectivity towards flavonoids (95.4 %) and the lowest rejection of sugar compounds (48.7 %) (Conidi et al. 2011).

The same authors evaluated the performance of different polymeric spiral-wound NF membranes (with MWCO of 250–1000 Da) in the treatment of pressed liquors obtained from pigmented orange peels for the separation of phenolic compounds from sugars. The results indicated that the average rejection selectivity towards sugars was reduced by increasing the MWCO of the selected membranes, while rejection of anthocyanins remained higher than 89 % for all the NF membranes investigated (Conidi et al. 2012).

The concentration of anthocyanins from roselle extract (*Hibiscus sabdariffa* L.) using flat-sheet UF and NF membranes with MWCO of 2–150 kDa was investigated by Cissé et al. (2011). The authors suggested that all membranes with nominal MWCO equal to or less than 20 kDa could be used for concentrating anthocyanins. Higher retention of anthocyanins was observed for the NF

membranes. In addition, at average transmembrane pressures, these membranes exhibited similar permeate fluxes when compared with UF membranes.

NF has also been found to be effective for the concentration of anthocyanins from ultrafiltered aqueous extract of aronia berries (Gilewica-Łukasik et al. 2007). The retention of anthocyanins was almost complete in the presence of sodium sulfate (IV) (rejection higher than 99 %), while the filtration of pure aronia solution or that containing sulfate (VI) was not as effective (rejection of 91–95 %).

The fractionation of phenolic compounds with membrane-based separation processes is another topic of interest. Santamaria et al. (2002) proposed sequential filtration with polymeric NF and UF membranes to fractionate proanthocyanidins from winery waste extracts on the basis of their molecular weight. The proposed process, depicted in Fig. 2.5, allowed the production of proanthocyanic fractions with different degrees of polymerization.

Grape anthocyanins were fractionated using flat-sheet PVDF UF membranes with MWCO of 10 to 1000 kDa. Polymeric forms were retained by membranes with MWCO of less than 100 kDa, while monomers were recovered in the permeate fractions (Kalbasi and Cisneros-Zevallos 2007). UF membranes with MWCO of 10 kDa also allowed separation of low molecular weight tannins from

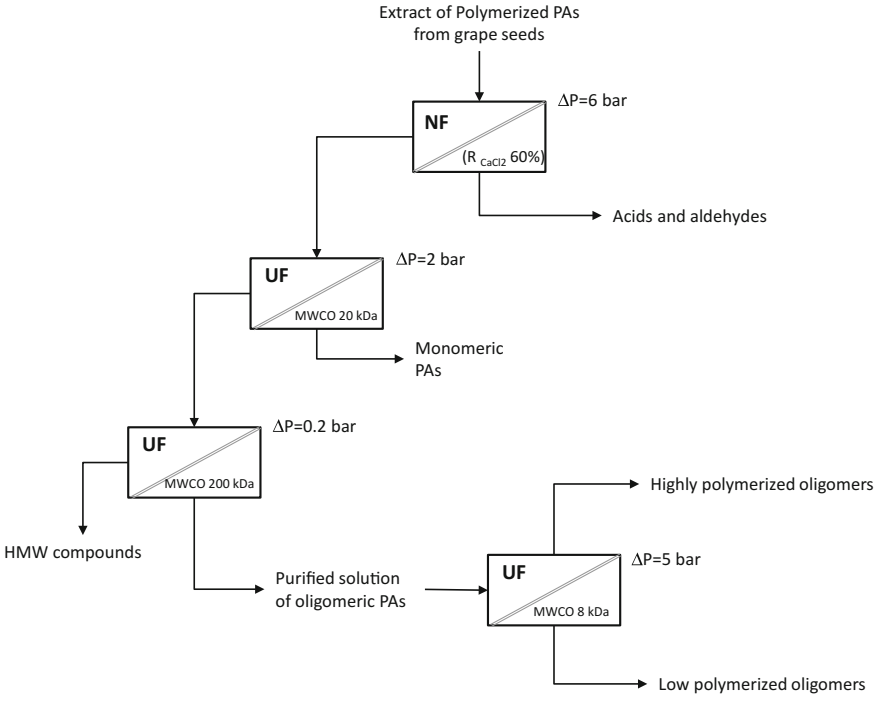

Fig. 2.5 Fractionation of proanthocyanidins (PAs) by integrated membrane operations (*HMW* High molecular weight; *MWCO* Molecular weight cut-off; *R* Rejection) (adapted from Santamaria et al. 2002)

high molecular weight phenolic compounds in the treatment of crude extract of persimmon (a plant belonging to the Ebenaceae family that is widespread in China, Japan and Korea) (Gu et al. 2008).

MF and UF membranes are also an effective tool for the separation of oligosaccharides and low molecular weight impurities from high molecular weight compounds (Li and Chase 2010). Fructo-oligosaccharides, which are widely recognized as functional food ingredients, can be purified and concentrated using NF membranes (Li et al. 2004) or low-MWCO UF membranes. Thin-film polymeric membranes with MWCO of 1 to 8 kDa were used to purify xylo-oligosaccharides from an almond shell hydrolysate (Nabarlatz et al. 2007). Selectivity was maximized by operating at low pressures, and was increased by reducing the MWCO of the membrane.

Tubular polymeric UF membranes (ESP04, PCI Membranes, Poland) and ceramic monolithic NF membranes (Kerasep Nano, Novasep, France) with MWCO of 4 and 1 kDa, respectively, were found to be effective for the purification of xylo-oligosaccharides from monosaccharides and salts from rice husk xylan (Vegas et al. 2008). NF membranes with MWCO of 150 to 300 Da were also proven to be efficient for the recovery of xylose from hemicellulose hydrolysates. Considerable xylose purification was achieved in short-term filtrations, which produced permeate streams containing 78–82 % xylose as total dry solids (Sjöman et al. 2008).

The purification of fructo-oligosaccharides from a mixture of sugars with different NF membranes was investigated by Kuhn et al. (2011). Specifically, the authors evaluated the performance of PA and PES flat-sheet membranes with MWCO over a range of 150 to 1000 Da in both dead-end and tangential-flow configurations. The best results were obtained with the NP030 membrane (Microdyn-Nadir, Germany) with an MWCO of 400 Da, which showed 66 % retention selectivity towards fructo-oligosaccharides, while retention selectivity towards glucose, fructose and sucrose was 18, 15 and 24 %, respectively. DF tests with this membrane produced a concentrate containing 80 % fructo-oligosaccharides.

2.5.1 Soy Protein Extracts

UF represents a valid alternative to the use of isoelectric precipitation for the production of soy protein isolates or concentrates from soy protein extracts. The use of UF allows the recovery of all solubilized proteins, avoiding the formation of whey-like products and resulting in increased protein recovery (Nichols and Cheryan 1981). In addition, undesirable compounds such as oligosaccharides (sucrose, raffinose and stachyose) and phytic acid can be selectively separated from the proteins through selection of optimal operating parameters and membrane types (Kumar et al. 2003).

The combination of UF and DF results in high phytic acid removal and high levels of protein purification. Phytic acid removal is affected by pH: high removal percentages have been observed within a pH range of 5–6.7 (Ali et al. 2010). UF

membranes with MWCO of 50 kDa represent the best option for obtaining high values of permeate fluxes, high protein rejection and high removal of oligosaccharides (Skorepova and Moresoli 2007). In addition, the use of UF membranes with MWCO of 5–30 kDa enables the retention of isoflavones, widely recognized for their anticarcinogenic properties due to their complexation with proteins (Singh 2007). In contrast, in the conventional isoelectric precipitation processes, most of the isoflavones remain soluble after the precipitation step.

The main functional properties of protein isolates (solubility, ability to emulsify, ability to bind water or fat and ability to form foams or gels) obtained by a combination of UF and DF were higher than those obtained with traditional isoelectric precipitation processes (Manak et al. 1980).

Soy protein isolates can also be used as a source of peptides of interest for nutraceutical applications. UF membranes compete with other technologies such as chromatography and ion-exchange resins for use in the fractionation and purification of specific peptides. In particular, soy protein hydrolysates can be subjected to a sequential treatment with UF membranes of increasing MWCO values (i.e. from 5 to 100 kDa) in order to produce different soy peptide fractions (Deeslie and Cheryan 1991).

Wastewater from the manufacture of soybeans contains valuable compounds such as proteins and sugars; their recovery appears to hold great potential for reducing the pollution load of produced effluents while simultaneously utilizing valuable resources.

The integration of pressure-driven membrane operations can be exploited for the extraction of proteins, oligosaccharides and isoflavones from yellow bean product wastewater (Jiang and Wang 2013). UF membranes retain soybean proteins, and the concentrated solution can be spray-dried to obtain a pure soybean protein powder. Soy isoflavones in the UF permeate are adsorbed on weak polar macroporous resins and then eluted with ethanol. Soybean oligosaccharides in the resin effluent liquid can be concentrated using NF membranes, and the concentrated liquid can be used for preparing oligosaccharide powders. The final treatment of the NF permeate by RO produces pure water through the removal of inorganic salts.

Soybean soaking water contains on average of 0.08 % (w/w) crude proteins and 0.02 % (w/w) carbohydrates. Their COD (typically higher than 10 g/L) must be properly reduced for purposes of reuse. The performance of a combined NF-RO membrane system for separating and recovering soluble materials from soybean soaking water was investigated by Guu et al. (1997). The pretreated water (after sedimentation and centrifugation) was nanofiltered through a spiral-wound membrane module (NF40-2514A, FilmTec/DOW Chemical Co.) up to a weight concentration ratio (WCR) of 7. The NF permeate was then subjected to an RO process using a spiral-wound membrane (BW30-2514, FilmTec/DOW Chemical Co.) up to a WCR of 6. Both processes were operated at 30 °C and a Δp of 25 bar. NF and RO retentates were inoculated with probiotic cultures of *Lactobacillus acidophilus* CCRC 10695 and *Bifidobacterium longum* CCRC 11847 in order to assess the possibility of producing lactic acid by fermenting soluble materials (Fig. 2.6). The combination of the two selected cultures produced up to 7.5 g/L of organic acids,

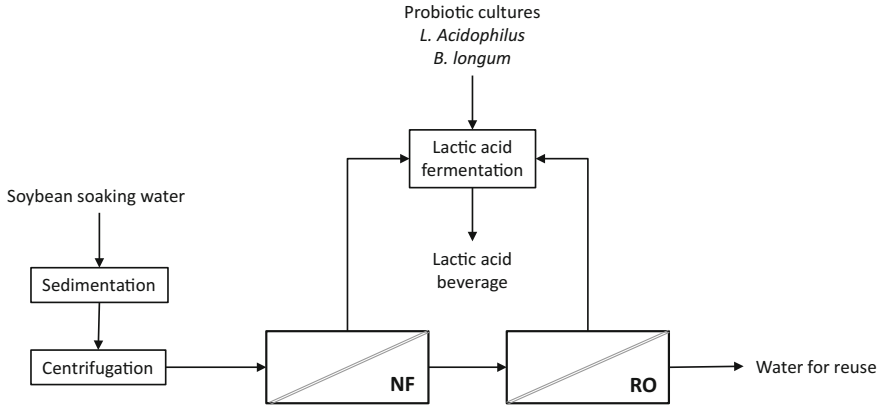

Fig. 2.6 Recovery of soluble compounds and water from soybean soaking water (adapted from Guu et al. 1997)

represented mainly by lactic acid, after 48 h of fermentation at pH 5.5 and a temperature of 37 °C. With this process, the fermentation broth after centrifugation and pasteurization is able to be used for the formulation of lactic acid beverages, while the RO permeate can be reused within the factory for cleaning and soaking purposes.

Spiral-wound NF and RO membranes have also been used for concentration of oligosaccharides (mainly sucrose, raffinose and stachyose) from steamed soybean wastewater after the removal of high molecular weight proteins by UF (Matsubara et al. 1996). Operating in a batch concentration mode, concentrated solutions containing 22 % (w/v) and 10 % (w/v) of oligosaccharides were obtained by RO and NF processes, respectively. The COD of the treated effluent (8400–8700 mg/L) was reduced by 99.6 and 98.1 %, respectively.

2.5.2 *Olive Mill Wastewater*

The procedures for extraction of olive oil from olives (press extraction, two-phase and three-phase centrifugation systems) produce large amounts of polluted liquid waste, known as olive mill wastewater (OMW) or vegetation water. It is a dark acidic liquid containing organic substances (sugars, tannins, organic acids, nitrogen-containing substances, and phenolic compounds including polyphenols, polyalcohols, pectins and lipids) and minerals. COD and BOD are found in a range of 40–220 and 35–110 g/L, respectively (Niaounakis and Halvadakis 2006). Several physicochemical, biological and combined processes, including centrifugation, membrane filtration, flocculation, adsorption, oxidation, and aerobic and anaerobic treatments, have been proposed for reducing the pollution load of these wastes. If properly managed, however, OMW represents an inexpensive and

convenient source of polyphenols, water-soluble compounds widely recognized for their anti-inflammatory, antimicrobial and antioxidant activity (Obied et al. 2005). These properties are reflected in the high market price and great demand for OMW in pharmaceutical, cosmetic and nutrition applications. According to the 'olive tree biorefinery' concept recently proposed by Federici et al. (2009), the recovery of phenolic compounds from OMW represents an efficient tool for valorization of the olive oil extraction residues.

Several methods have been proposed for the recovery of polyphenols from OMW, including liquid–liquid extraction (Kalogerakis et al. 2013), adsorption onto resins (Scoma et al. 2011), supercritical fluid extraction (Lafka et al. 2011) and fungal enzyme-aided extraction (Garcia Garcia et al. 2000). Most of these methods are expensive and present some drawbacks, such as the presence of residual solvents in the extract and the co-extraction of undesirable compounds.

Pressure-driven membrane operations, mostly in sequential form, meet the requirements for the recovery, purification and concentration of polyphenols from OMW, providing several advantages (low energy requirement, no additives, mild operating conditions, efficient separation and easy scale-up) over conventional technologies (Takaç and Karakaya 2009; Garcia-Castello et al. 2010; Mudimu et al. 2012).

Paraskeva et al. (2007) proposed a combination of UF and NF or RO membranes for a complete fractionation of OMW. In order to improve the performance of the UF membranes (multichannel ceramic membranes with 100 nm pores) and reduce their fouling, OMW was pre-filtered with an 80 μm PP screen. The UF process separated high molecular weight constituents including fats, lipids and suspended solid particles. A drastic reduction in turbidity was also observed independently of the applied Δp. At Δp values higher than 1.75 bar, the permeate fluxes were independent of the applied pressure. The NF treatment of the UF permeate with spiral-wound polymeric membranes (MWCO 200 Da) yielded a concentrated stream containing more than 95 % of the initial phenolic compounds. Better efficiency of the OMW treatment was achieved by applying RO (spiral-wound membrane modules 100 MWCO) after UF. Permeate fractions (75–80 % of the initial volume) from NF and RO treatments exhibited quality characteristics suitable for discharging in aquatic systems, according to EU regulations, or for irrigation.

An integrated membrane process for the selective separation of polyphenols from OMW was patented by ENEA in 2005 (Pizzichini and Russo 2005). In the proposed approach, depicted in Fig. 2.7, OMW was acidified at pH 3–4.5 in order to prevent oxidation of polyphenols, and was then subjected to enzymatic treatment with pectinase to hydrolyze cellulose, hemicellulose and pectin. The degradation products were separated by centrifugation to obtain a partially clarified liquid fraction, which was then subjected to a first MF step carried out with ceramic membranes (pore size of 0.1–1.4 μm). The MF permeate was fed to a UF unit equipped with spiral-wound polymeric membranes (PS, PES, PA or CA) with MWCO ranging from 1 to 20 kDa. The DF process, which consists of the addition of water to MF and UF concentrates, can increase the recovery of phenolic compounds in the permeate fractions. Solid residues from the centrifugation step can be

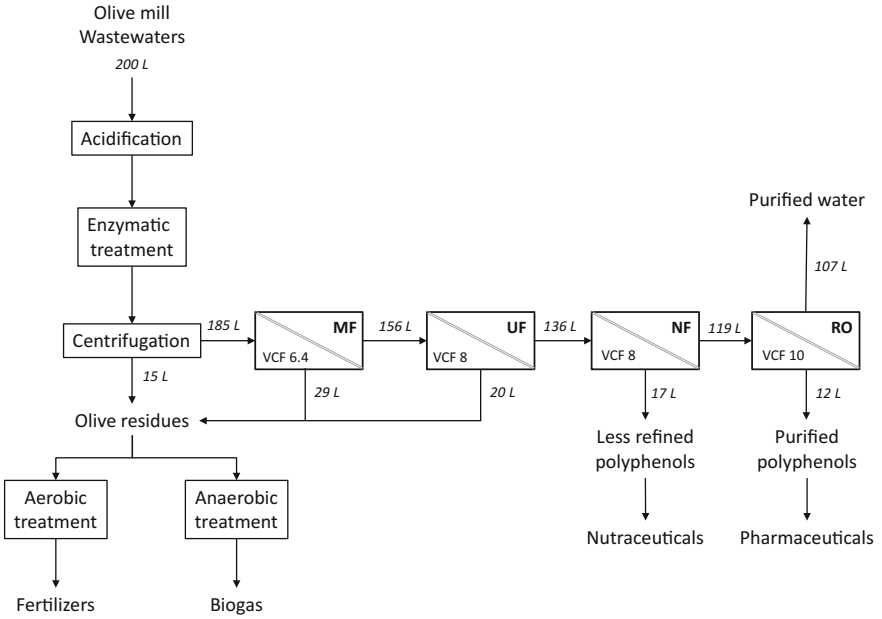

Fig. 2.7 Proposed process scheme for the recovery of water and phenolic compounds from OMW (adapted from Pizzichini and Russo 2005)

added to MF and UF retentates and used as a basis for compost production or subjected to aerobic or anaerobic treatment. The UF permeate is subjected to an NF treatment with spiral-wound polymeric membranes (PA or nylon, MWCO 150–250 Da), producing a permeate containing the maximum amount of hydroxytyrosol and other polyphenols of biomedical interest, and a retentate containing various phenolic compounds (including tyrosol, coumaric acid, hydroxytyrosol and caffeic acid), which can be used for the production of functional foods or cosmetics. The NF permeate is subjected to a final RO treatment using spiral-wound polymeric membranes made of composite PA, producing a concentrated liquid enriched in purified polyphenols of interest for pharmaceutical applications and ultrapure water suitable for beverage formulations.

The combination of UF and NF polymeric membranes for the recovery of phenolic compounds from OMW was recently investigated at the laboratory level by Cassano et al. (2013). OMW, produced according to a three-phase centrifugation process, was pretreated with hollow fiber UF membranes (PVDF, pore size 0.02 μm, Toray) in order to remove most of suspended solids. The UF permeate was then subjected to a second UF step performed with 1000-Da flat-sheet membranes (Etna 01PP composite fluoropolymer, MWCO 1 kDa, Alfa Laval, Sweden). A concentrated phenolic solution was obtained by treating the UF permeate with a spiral-wound NF membrane (NF90, Filmtec/Dow, USA). Analyses of phenolic compounds, total antioxidant activity (TAA) and total organic carbon (TOC) in feed

and permeate fractions for each process are summarized in Table 2.1. The rejection selectivity of the UF and NF membranes towards these compounds is also included. Phenolic compounds were recovered in the UF permeate of the first UF step; most of the organic substances were separated from phenolic compounds in the second UF step, as shown by the high rejection selectivity of the Etna 01PP membrane towards TOC.

Table 2.1 Analyses of polyphenols, total antioxidant activity (TAA) and total organic compounds (TOC) in samples of olive mill wastewaters treated by UF and NF membranes (adapted from Cassano et al. 2013)

Membrane type	Parameter	Feed	Permeate	Rejection (%)
UF (PVDF, 0.02 mm)	Polyphenols (mg GAE/L)	1409	1033	26.7
	TAA (mM Trolox)	3000	2750	8.3
	TOC (mg/L)	13,436	8898	33.7
UF (composite fluoro-polymer, 1 kDa)	Polyphenols (mg GAE/L)	960.8	654.6	31.8
	TAA (mM Trolox)	2695	1750	35.0
	TOC (mg/L)	9000	2500	72.1
NF (polyamide, 150 Da)	Polyphenols (mg GAE/L)	624.7	43.3	93.0
	TAA (mM Trolox)	1825	125	93.0
	TOC (mg/L)	2800	95	96.4

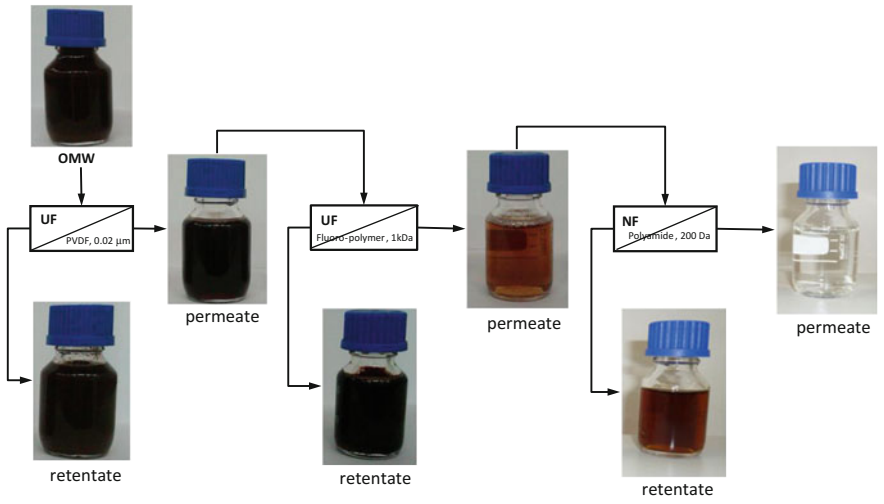

Fig. 2.8 Permeate and retentate fractions from an integrated membrane process for the recovery of polyphenols from OMW (Cassano et al. 2013) (with permission of Elsevier)

Permeate and retentate samples from the fractionation of OMW with the selected membranes are shown in Fig. 2.8.

According to the proposed process, three main fractions are produced: (i) a concentrated solution (retentate of both UF processes) containing high molecular weight organic substances, which can be subjected to anaerobic digestion for the production of biogas; (ii) a concentrated solution (NF retentate) rich in polyphenolic compounds suitable for cosmetic, food and pharmaceutical industries as liquid, frozen, dried or lyophilized formulations; and (iii) a water stream (NF permeate), which can be reused as processing water, membrane cleaning solution, or in the DF step to increase the yield of polyphenols in UF permeates.

2.6 Conclusions and Future Trends

Pressure-driven membrane operations offer a wide range of advantages over conventional technologies for the separation, purification and concentration of high-added-value compounds from food products and food processing wastewater. A high degree of selectivity, better control of the production process, water savings and low energy costs are additional advantages.

The demand for products with greater nutritional value, the development of environmentally friendly processing procedures and the increasingly urgent need to reduce water consumption are all factors that will contribute to the continued growth of the membrane market. In addition, the possibility of combining different membrane operation units within integrated membrane systems or with conventional separation technologies offers interesting new perspectives for redesigning traditional flow sheets of the agro-food industry within the context of process intensification strategies.

The optimization of procedures for reducing membrane fouling, including the development of new membranes and membrane modules less prone to fouling, appears to be a promising area for future investigations aimed at improving the productivity, selectivity and durability of membrane systems. Additional challenges concern the development of functional membranes with specific functional groups grafted onto their surface, conferring high selectivity for target substances and low fouling.

References

Afonso MD, Bórquez R (2002) Review of the treatment of a seafood processing wastewater and recovery of proteins therein by membrane separation processes—prospects of the ultrafiltration of wastewater from the fish meal industry. Desalination 142:29–45

Afonso MD, Ferrer J, Bórquez R (2004) An economic assessment of proteins recovery from fish meal effluents by ultrafiltration. Trends Food Sci Technol 15:506–512

Aguiar Prudêncio AP, Prudêncio ES, Castanho Amboni RDM et al (2012) Phenolic composition and antioxidant activity of the aqueous extract of bark from residues from mate tree (*Ilex paraguariensis* St. Hil.) bark harvesting concentrated by nanofiltration. Food Bioprod Process 90:399–405

Ali F, Ippersiel D, Lamarche F et al (2010) Characterization of low-phytate soy protein isolates produced by membrane technologies. Innov Food Sci Emerg Tech 11:162–168

Atra R, Vatai G, Bekassy-Molnar E et al (2005) Investigation of ultra- and nanofiltration for utilization of whey protein and lactose. J Food Eng 67:325–332

Baker WR (2000) Membrane technologies and applications. Mc-Graw-Hill Companies, New York

Bhattacharya S, Hwang ST (1997) Concentration polarization, separation factor, and Peclet number in membrane processes. J Membr Sci 132:73–90

Boerlage SFE, Kennedy M, Tarawneh Z et al (2004) Development of the MFI-UF in constant flux filtration. Desalination 161:103–113

Cassano A, Conidi C, Giorno L et al (2013) Fractionation of olive mill wastewater by membrane separation techniques. J Hazard Mater 248–249:185–193

Cissé M, Vaillant F, Pallet D et al (2011) Selecting ultrafiltration and nanofiltration membranes to concentrate anthocyanins from roselle extract (*Hibiscus sabdariffa* L.). Food Res Int 44:2607–2614

Conidi C, Cassano A, Drioli E (2011) A membrane-based study for the recovery of polyphenols from bergamot juice. J Membr Sci 375:182–190

Conidi C, Cassano A, Drioli E (2012) Recovery of phenolic compounds from orange press liquor by nanofiltration. Food Bioprod Process 90:867–874

Daufin G, Escudier JE, Carrère H et al (2001) Recent and emerging applications of membrane processes in the food and dairy industry. Trans IChemE 79:89–102

Deeslie WD, Cheryan M (1991) Fractionation of soy protein hydrolysates using ultrafiltration membranes. J Food Sci 57:411–413

Denis C, Massé A, Fleurence J et al (2009) Concentration and pre-purification with ultrafiltration of a R-phycoerythrin solution extracted from macro-algae *Grateloupia turuturu*: process definition and up-scaling. Sep Purif Technol 69:37–42

Di Donna L, De Luca G, Mazzotti F et al (2009) Stain-like principles of Bergamot fruit (Citrus bergamia): isolation of 3-hydroxymethylglutaryl flavonoid glycosides. J Nat Prod 72:1352–1354

Diaz-Reinoso B, Moure A, Dominguez H et al (2009) Ultra- and nanofiltration of aqueous extracts from distilled fermented grape pomace. J Food Eng 91:587–593

Diaz-Reinoso B, Moure A, Dominguez H et al (2011) Membrane concentration of antioxidants from *Castanea sativa* leaves aqueous extracts. Chem Eng J 175:95–102

Federici F, Fava F, Kalogerakis N et al (2009) Valorisation of agro-industrial by-products, effluents and waste: concept, opportunities and the case of olive mill wastewaters. J Chem Technol Biotechnol 84:895–900

Garcia-Castello E, Cassano A, Criscuoli C et al (2010) Recovery and concentration of polyphenols from olive mill wastewaters by integrated membrane system. Water Res 44:3883–3892

Garcia Garcia I, Jimenez Peña PR, Bonilla Venceslada JL et al (2000) Removal of phenol compounds from olive mill wastewater using *Phanerochaete chrysosporium*, *Aspergillus niger*, *Aspergillus terreus* and *Getrichum candidum*. Process Biochem 35:751–758

Gernigon G, Schuck P, Jeantet R (2011) Demineralization. In: Fuquay JW, Fox PF, McSweeney PLH (Eds) Encyclopedia of dairy science. Elsevier, London, pp 738–743

Gilewica-Łukasik B, Koter S, Kurzawa J (2007) Concentration of anthocyanins by the membrane filtration. Sep Purif Technol 57:418–424

Gonzáles MI, Alvarez S, Riera FA et al (2008) Lactic acid recovery from whey ultrafiltrate fermentation broths and artificial solutions by nanofiltration. Desalination 228:84–96

Guizard C, Amblard P (2009) Current status and prospects for ceramic membranes application. In: Pabby K, Rizvi S, Sastre A (eds) Handbook of membrane separation: chemical, pharmaceutical, food and biotechnological applications. Taylor Francis Group, Boca Raton, pp 139–179

Gu HF, Li CM, Xu YJ (2008) Structural features and antioxidant activity of tannin from persimmon pulp. Food Res Int 41:208–217

Guu JK, Chiu CH, Young JK (1997) Processing of soybean soaking water with a NF-RO membrane system and lactic acid fermentation of retained solutes. J Agric Food Chem 45:4096–4100

Jiang B, Wang J (2013) Method for extracting soy protein, oligosaccharide and isoflavone from soybean wastewater by one-step process. Chinese patent CN103265614-A

Kalbasi A, Cisneros-Zevallos L (2007) Fractionation of monomeric and polymeric anthocyanins from concord grape (*Vitis labrusca* L.) juice by membrane ultrafiltration. J Agric Food Chem 55:7036–7042

Kalogerakis N, Politi M, Foteinis S et al (2013) Recovery of antioxidants from olive mill wastewaters: a viable solution that promotes their overall sustainable management. J Environ Manage 128:749–758

Karasau K, Glennon N, Laurence ND et al (2010) A comparison between ceramic and polymeric membrane systems for casein concentrate manufacture. Int J Dairy Technol 63:284–289

Kaur C, Kapoor HC (2001) Antioxidants in fruits and vegetables—the millennium's health. Int J Food Sci Technol 36:703–725

Kiers G (2001) Feta in liquid form. Dairy Ind Int 66:21–21

Korhonen H, Syväoja EL, Vasara E et al (1998) Pharmaceutical composition, comprising complement proteins, for the treatment of *Helicabacter* infections and a method for the preparation of the composition. PCT Patent Application WO98/00150

Kuhn RC, Palacio L, Prádanos P et al (2011) Selection of membranes for purification of fructooligosaccharides. Desalin Water Treat 27:18–24

Kumar NSK, Yea MK, Cheryan M (2003) Soy protein concentrates by ultrafiltration. J Food Sci 68:2278–2283

Lafka T, Lazou A, Sinanoglou V et al (2011) Phenolic and antioxidant potential of olive oil mill wastes. Food Chem 125:92–98

Li W, Li J, Chen T et al (2004) Study on nanofiltration for purifying fructo-oligosaccharides I. Operation modes. J Membr Sci 245:123–129

Li J, Chase HA (2010) Applications of membrane technique for purification of natural products. Biotechnol Lett 32:601–608

Mameri N, Abdessemed D, Belhocine D et al (1996) Treatment of fishery washing water by ultrafiltration. J Chem Technol Biotechnol 67:169–175

Manak LJ, Lawhon JT, Lusas EW (1980) Functioning potential of soy, cottonseed, and peanut protein isolates produced by industrial membrane systems. J Food Sci 45:236–245

Market Report: Global Membrane Technology Market (2013) Ratingen. http://www.acmite.com/

Massé A, Vandanjon L, Jaouen P et al (2008) Upgrading and pollution reduction of fishing industry process-waters by membrane technology. In: Bergé JP (ed) Added value to fisheries wastes. Transworld Research Network, Kerala, pp 81–99

Matsubara Y, Iwasaki K, Nakajima M et al (1996) Recovery of oligosaccharides from steamed soybean waste water in Tofu processing by reverse osmosis and nanofiltration membranes. Biosci Biotech Biochem 60:421–428

Mello BCBS, Cunha Petrus JC, Hubinger MD (2010) Concentration of flavonoids and phenolic compounds in aqueous and ethanolic propolis extracts through nanofiltration. J Food Eng 96:533–539

Mudimu OA, Peters M, Brauner F et al (2012) Overview of membrane processes for the recovery of polyphenols from olive mill wastewater. Am J Environ Sci 8:195–201

Nabarlatz D, Torras C, Garcia-Valls R et al (2007) Purification of xylo-oligosaccharides from almond shells by ultrafiltration. Sep Purif Technol 53:235–243

Nawaz H, Shi J, Mittal GS et al (2006) Extraction of polyphenols from grape seeds and concentration by ultrafiltration. Sep Purif Technol 48:176–181

Negrão Murakami AN, de Mello Castanho Amboni RD, Prudêncio ES et al (2011) Concentration of phenolic compounds in aqueous mate (*Ilex paraguariensis* A. St. Hil) extract through nanofiltration. LWT—Food Sci Technol 44:2211–2216

Niaounakis M, Halvadakis CP (2006) Olive processing waste management literature review and patent survey. Waste Manage Series 5:23–64
Nichols DJ, Cheryan M (1981) Production of soy isolates by ultrafiltration: factors affecting yield and composition. J Food Sci 46:367–372
Obied HK, Allen MS, Bedgood DR et al (2005) Bioactivity and analysis of biophenols recovered from olive mill waste. J Agric Food Chem 53:823–837
Paraskeva CA, Papadakis VG, Tsarouchi E et al (2007) Membrane processing for olive mill wastewater fractionation. Desalination 213:218–229
Piot M, Fauquant J, Madec MN et al (2004) Preparation of "serocolostrum" by membrane microfiltration. Lait 84:333–342
Pizzichini M, Russo C (2005) Process for recovering the components of olive mill wastewater with membrane technologies. Int. Patent WO 2005/123603
Prodanov M, Garrido I, Vacas V et al (2008) Ultrafiltration as alternative purification procedure for the characterization of low and high molecular-mass phenolics from almond skins. Anal Chim Acta 609:241–251
Ramakrishna S, Zuwei M, Matsuura T (2011) Polymer membranes in biotechnology—preparation, functionalization and application. Imperial College Press, London
Rosenberg M (1995) Current and future applications for membrane processes in the dairy industry. Trends Food Sci Technol 6:12–19
Santamaria B, Salazar G, Beltrán S et al (2002) Membrane sequences for fractionation of polyphenolic extracts from defatted milled grape seeds. Desalination 148:103–109
Schäfer AI, Fane AG, Waite TD (2000) Fouling effects on rejection in the membrane filtration of natural waters. Desalination 131:215–224
Schwinge J, Neal PR, Wiley DE et al (2004) Spiral wound modules and spacers, review and analysis. J Membr Sci 242:129–153
Scoma A, Bertin L, Zanaroli G et al (2011) A physicochemical-biotechnological approach for an integrated valorization of olive mill wastewater. Bioresour Technol 102:10273–10279
Sherwood RK, Pigford RL, Wilke CR (1975) Mass transfer. McGraw Hill, New York
Singh N (2007) Process for producing a high solubility, low viscosity, isoflavone-enriched soy protein isolate and the products thereof. US Patent 7,306,821 B2
Singh V, Jain PK, Das C (2013) Performance of spiral wound ultrafiltration membrane module for with and without permeate recycle: experimental and theoretical consideration. Desalination 322:94–103
Sjöman E, Mänttäri M, Nyström M et al (2008) Xylose recovery by nanofiltration from different hemicellulose hydrolyzate feeds. J Membr Sci 310:268–277
Skorepova J, Moresoli C (2007) Carbohydrate and mineral removal during the production of low-phytate soy protein isolate by combined electroacidification and high shear tangential flow ultrafiltration. J Agric Food Chem 55:5645–5652
Sondhi R, Bhave R (2001) Role of backpulsing in fouling minimization in crossflow filtration with ceramic membranes. J Membr Sci 186:41–52
Sotto A, Arsuaga JM, Van der Bruggen B (2013) Sorption of phenolic compounds on NF/RO membrane surfaces: influence on membrane performance. Desalination 309:64–73
Strathmann H, Giorno L, Drioli E (2006) An introduction to membrane science and technology. Consiglio Nazionale delle Ricerche, Roma
Susanto H, Feng Y, Ulbricht M (2009) Fouling behaviour of aqueous solutions of polyphenolic compounds during ultrafiltration. J Food Eng 91:333–340
Takaç S, Karakaya A (2009) Recovery of phenolic antioxidants from olive mill wastewater. Rec Patents Chem Eng 2:230–237
Tamime AY, Robinson RK, Kiers G (2006) Industrial manufacture of feta-type cheeses In: Tamime AY (Ed) Brined cheeses. Blackell Publishing Ltd, Oxford, pp 77–116
Tylkowski B, Trusheva B, Bankova V et al (2010) Extraction of biologically active compounds from propolis and concentration of extract by nanofiltration. J Membr Sci 348:124–130

Tylkowski B, Tsibranska I, Kochanov R et al (2011) Concentration of biologically active compounds extracted from *Sideritis ssp.* L. by nanofiltration. Food Bioprod Process 89:307–314

Vegas R, Moure A, Dominguez H, Parajó JC, Alvarez JR, Luque S (2008) Evaluation of ultra- and nanofiltration for refining soluble products from rice husk xylan. Bioresour Technol 99:5341–5351

Vyas HK, Bennett RJ, Marshall AD (2000) Influence of feed properties on membrane fouling in crossflow microfiltration of particulate suspensions. Int Dairy J 10:855–861

Wu D, Howell JA, Field RW (1999) Critical flux measurement for model colloids. J Membr Sci 152:89–98

Yorgun MS, Akmehmet Balcioglu I, Saygin O (2008) Performance comparison of ultrafiltration, nanofiltration and reverse osmosis on whey treatment. Desalination 229:204–216

Zidney AL (1998) Protein separations using membrane filtration: new opportunities for whey fractionation. Int Dairy J 8:243–250

Chapter 3
Industrial Production of Active Probiotics for Food Enrichment

Sai Swaroop Dalli, Bijaya K. Uprety and Sudip K. Rakshit

Abstract Functional foods are defined as foods that have health benefits beyond their inherent nutritional value. The incorporation of probiotics in food products is one of the most popular forms of such products and acceptable to most consumers. In this chapter, various industrial probiotic products are discussed, including the type of microorganisms used and the production process. Details of processing conditions and choice of probiotics for retaining the viability of the microorganism through production are included. Advanced non-food application of probiotics and the potential for such products are also presented.

Keywords Probiotics · Fortified products · Stability and safety · Non-food applications

3.1 Introduction to Probiotics

Functional foods are foods that have a potentially positive effect on health beyond basic nutrition. Probiotics are living bacteria and yeasts that are incorporated into food products because they help to maintain the microbial flora in the gut, which in turn results in good overall health. An estimated 500 to 1000 species of microorganisms inhabit the digestive system. Maintaining a good distribution of beneficial microorganisms prevents the inhabitation of pathogenic microbes and is also known to improve the immune response.

An ideal probiotic microbe must be of human origin, with beneficial physiological effects, and should be generally regarded as safe (GRAS) (Singh et al. 2011). An efficient probiotic should have important properties including good stability under storage and distribution conditions, and should be non-pathogenic, non-toxic, sustainable in the host body, with effective adhesion, resistance to low

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pH and bile salts in the gut, and should have good activity, with beneficial effects on the host (e.g. enhancing immunity) (Fuller 1989).

The common criteria for probiotics used in industry are as follows:

- **Biosafety**: Microbes should be generally regarded as safe (GRAS) (e.g. *Lactobacillus, Bifidobacterium, Enterococcus*).
- **Origin**: Microbes used in probiotics should preferably be autochthonous in origin.
- **Resistance in in vitro and in vivo conditions**: Microbes should be tolerant to the defense mechanisms, pH, bile and pancreatic conditions in the host body.
- **Adherence**: Microbes should adhere to the intestinal epithelium and colonize to survive for longer periods.
- **Antimicrobial activity**: An important criteria for probiotic organisms is that they are effective in protecting the host against pathogens. Therefore, they should exhibit certain antagonistic properties. For example, *Lactobacillus* sp., a widely used microbe in probiotics, produces inhibitors (e.g. bacteriocins), reduces redox potential, produces hydrogen peroxide, decreases pH through the production of lactic acid and protects the human host against harmful organisms (Kosin and Rakshit 2006).

Over the past two decades, a considerable amount of research and development has been focused on probiotics and their inclusion in many traditional foods. Consumer acceptance has resulted in the availability of large numbers of probiotic food products and their industrial production. The present chapter reviews the major industrial probiotic products, including the type of probiotics involved, the production processes, and the related stability and food safety issues. The application of probiotics in non-food applications in feed, medicine, aquaculture and veterinary products is also covered. Table 3.1 provides a list of common microbes, their application and probiotic properties.

3.2 Industrial Probiotic Products

There is an increased awareness of the health benefits of dietary food and functional foods with probiotics. According to market reports, up to 93 % of consumers in North America believe that the risk of disease can be reduced by the consumption of functional foods (Champagne and Møllgaard 2008). Industries are competing for the production of efficient probiotic functional foods, as high growth rates and low processing costs make them good candidates for industrial production. In 2011, the market for probiotics products was valued at $27.9 billion, and this is expected to increase substantially, by 6.8 % annually, reaching $44.9 billion by 2018 (Chr. Hansen 2013). The Asia-Pacific region is currently experiencing the highest rate of growth in probiotics production, followed by Europe (Chr. Hansen 2013). Some of the dairy-based industrial probiotic strains in use in a variety of market products are listed in Table 3.2.

Functional dairy-based foods can be divided into fortified dairy and whey (protein)-based products (Özer and Kirmaci 2010).

Table 3.1 Common probiotic bacteria, properties and applications (BC Diary 2015)

Bacteria	Properties	Use	Potential probiotic effects
Lactobacillus acidophilus	Acid-resistant Grows slowly and is less viable in fermented products	Used in acidophilus milk and in kefir; may be used in yogurt	Fights intestinal infection and may prevent colon cancer. Reduces intestinal transit time
Lactobacillus GG	Good adherence. Colonies do not last long; therefore, recommended to consume a few times a week Minimum levels for colonization are • 10^8 cells in milk • 10^9 cells in fermented milk or enteric tablets • 10^{10} cells in gelatin capsules	Some new fermented dairy products using LGG are available in Europe	Inhibits pathogens. Reduces tumor development Prevents traveler's diarrhea, antibiotic-associated diarrhea and infant diarrhea
Lactobacillus casei	Some strains are acid-tolerant Does not colonize	Used in kefir and many cheeses including cheddar, also used in some new yogurt-like products	Induces the activity of intestinal microflora. Decreases incidence and duration of diarrhea Induces levels of immunoglobulins, γ-interferon and phagocytic activity. Reduces the risk of colon cancer
Bifidobacterium	Some strains are acid-tolerant, but unclear on adherence and colonization Produces both lactic acid and acetic acid	Can be used in yogurt	Restricts growth of pathogens Prevents and cures diarrhea Reduces intestinal transit time and may reduce colon cancer Induces production of secretory immunoglobulin

3.2.1 Fortified Dairy Products

Dairy products are considered to be good vectors for the delivery of probiotics to humans, because of their inherent characteristics that favor probiotic growth and make

Table 3.2 Some of the prevailing probiotic products in the market

Brand name	Mode of delivery	Strains	Scope	Producer	Effect	Recommended dose	Reference
Bifidus Regularis® Activia	Yogurt	*Bifidobacterium lactis* CNCM I-2494	Human	Dannon (USA)	Reduces irritable bowel syndrome (IBS) symptoms, reduces GI discomfort	10^{10} CFU × 2/day	(Activia; Guyonnet et al. 2007; Aureli et al. 2011; Agostini et al. 2012)
Actimel	Fermented milk	*L. casei* DN-114 001	Human	Danone (UK)	Prevention of *C. difficile* infection (CDAD) in adults	10^{10} CFU × 2/day	(Danon Actimel; Hickson et al. 2007; Aureli et al. 2011)
Yakult Light	Fermented dairy drink	*L. casei* Shirota	Human	Yakult (USA)	Reduces the incidence of antibiotic-associated diarrhea (AAD), positive effect on natural killer (NK) cell activity	6.5×10^9 CFU × 1/day	(Yakult; Activity et al. 2007; Wong et al. 2014)
Florastor®	Capsule (250 mg)	*Saccharomyces cerevisiae boulardii*	Human	Biocodex (Canada)	Prevention of AAD	250 mg ($\sim 5 \times 10^9$ CFU) × 2/day	(Sazawal et al. 2006; Florastor 2009; Aureli et al. 2011)
BioGaia Gastrus	Chewable tablets	*L. reuteri* 17,938, *L. reuteri* ATCC PTA 6475	Human	Biogaia (Sweden)	Treatment of *H. pylori* infection, acute infectious diarrhea in children	Each tablet (2×10^8 CFU)/day	(BioGaia; Francavilla et al. 2014; Szajewska et al. 2014)

(continued)

Table 3.2 (continued)

Brand name	Mode of delivery	Strains	Scope	Producer	Effect	Recommended dose	Reference
Culturelle	Capsule	*L. rhamnosus* GG (LGG)	Human	Valio Dairy (Finland)	Treatment of acute infectious diarrhea in children, AAD, irritable bowel syndrome, adjuvant in *H. pylori* eradication	Each capsule (1×10^{10} CFU)/day	(Aureli et al. 2011; Culturelle® 2013)
LIFE START®2	Goat milk (2.50 oz powder)	*Bifidobacterium infantis*	Human	Natren (USA)	Alleviation of irritable bowel syndrome, reduces risk of necrotizing enterocolitis	1 gm (2×10^{9} CFU)/day	(KeLATOX; Aureli et al. 2011; UC Davis 2014)
inner-ēco Coconut Water Probiotic Kefir	Fermented coconut water	*Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *diacetylactis, Leuconostoc mesenteroides* subsp. *cremoris, Lactobacillus kefiri, Kluyveromyces marxianus* var. *marxianus, Saccharomyces unisporus*	Human	inner-ēco (USA)	Promotes healthy microflora ecology in the GI tract, reduces sugar cravings	1 tbsp* (1×10^{11} CFU)/day	(Wegmans; Aureli et al. 2011; TrendMonitor 2013)
Enviva® PRO	Dry granules	*Bacillus subtilis* (3 strains)	Poultry	Danisco Animal Nutrition (UK)	Reduces coliform colonization in the gut, enhances immunity reduces nutritional stress in the gut	1.5×10^{5} CFU/g of feed	(Danisco; Lee et al. 2011)
Enviva® MPI	Fermented milk product	*L. rhamnosus*, *L. farciminis*	Piglets	Danisco Animal Nutrition (UK)	Supports piglet gut health, protects the growing pig against zoonotic bacteria such as *E. Coli*, *Clostridium*, *Brachyspira, Campylobacter jejuni*	5 g (5×10^{8} VFU)/kg of feed	(Bernardeau et al. 2009; Enviva 2013; Tareb et al. 2013)

(continued)

Table 3.2 (continued)

Brand name	Mode of delivery	Strains	Scope	Producer	Effect	Recommended dose	Reference
Life Products 10-G	Dry granules or oil	*Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus plantarum, Pediococcus acidilactici*	Dairy cattle	Life Products (USA)	Reduces the growth of *E. coli* and other pathogenic bacteria, improves immune system, improves milk productivity and feed consumption	1 g (2×10^9 CFU)/head/day	(Lifeproducts)
BACTOCELL® PA10	Lyophilized live bacteria	*Pediococcus acidilactici* MA 18/5 M	Shrimp & fish	LALLEMAND Inc. (Canada)	Supports piglet gut health, protects the growing pig against zoonotic bacteria such as *E. Coli*, *Clostridium*, *Brachyspira, Campylobacter jejuni*	**Salmonids and shrimp:** 100–1000 g/ton of complete feed **Marine fish:** 100–200 g/ton of feed (1 g = $1*10^{10}$ CFU)	(Lallemand; Castex et al. 2009)
Probiotics Spilac	Powder	*L. sporogenes*	Shrimp & fish	Guybro Chemical (India)	Improves growth, enhances biomass and immunity	**Fish:** 1 kg/ton of feed **Shrimp/prawn:** 2 kg/ton of mash feed, 5 kg/ton of pellet feed (1 kg = $33.2*10^9$ CFU)	(Guybro; Seenivasan et al. 2014)
SUKAFEED-B. Sub	Powder	*Bacillus subtilis*	Pigs, poultry, ruminants and aquatic animals	Sukahan (Weifang) Bio-Technology Co. LTD (China)	Improves feed efficiency and promotes digestion and absorption of nutrients in feed, enhances immune function, reduces stress, prevents diarrhea and dysentery	500–1000 g per ton of feed (1 g = 2×10^9 CFU)	(Sukahan; Soccol et al. 2013)

(continued)

Table 3.2 (continued)

Brand name	Mode of delivery	Strains	Scope	Producer	Effect	Recommended dose	Reference
P-Lact Plus	Powder	*Lactobacillus casei, Streptococcus faecium, Lactobacillus acidophilus, Lactobacillus lactis, Bacillus coagulans, Bacillus licheniformis, Saccharomyces cerevisiae*	Shrimp/prawn	Marine Aqua Technologies (India)	Enhances growth and body weight, maintains optimal health and immunity	**Regular use**: 10 g/kg of pellets **Stress/poor growth/disease condition:** 20 g/kg of pellets 10 days (kg = 4.5×10^5 CFU)	(PVS Laboratories Limited)

*tbsp: tablespoon; *L.*: *Lactobacillus*

them sustainable through the storage period. For the application of probiotics in dairy products, industries must focus on specific parameters (e.g. acidity, pH, dissolved oxygen content, redox potential and hydrogen peroxide effect) to meet essential requirements of probiotic properties (Akin 2005; Bais et al. 2006; Akin et al. 2007), health effects and regulations (Sanders and Klaenhammer 2001; Parvez et al. 2006). For good therapeutic effects, it is normally accepted that probiotic bacterial concentration in milk products should range from 10^6 to 10^7 colony-forming units per milliliter (CFU/mL). This can be maintained by reducing the dissolved oxygen level using microencapsulated cells (Ozer et al. 2005; Akin et al. 2007).

Probiotic dairy drinks were the first commercialized functional beverages, and are consumed worldwide in huge amounts in the form of a wide variety of products (Halliwell 2002; Hilliam 2004). Some of the commercial dairy-based probiotics and their manufacturers are listed in Table 3.3. *Lactobacillus rhamnosus* GG (LGG) is the most widely used probiotic bacterium in dairy industries because of its viability in the human gut and good acid tolerance. Some of the dairy product brands containing this bacteria are Gefilus, developed by Valio Ltd. (Finland), Aktifit, Biola, BioAktiv, YOMO, LGG+, Yoplait 360° and Kaiku Actif, which include milk, yogurt, buttermilk and drink products (Özer and Kirmaci 2010).

3.2.1.1 Probiotic Milk

Milk is the primary food through which probiotics can be delivered to the human body. The bacterium most widely used as a probiotic strain in milk is *Lactobacillus acidophilus*, because of its low growth rate and stability in milk. Another commonly used probiotic bacterium in such products is *Bifidobacterium bifidum*. In order to allow the growth of *L. acidophilus*, an acidic pH (5.5–6.0) must be maintained in the medium. However, the fermentation of milk often results in a drop in pH to below these levels, leading to a reduction in bacterial count.

Production Process

The production of *L. acidophilus* milk includes heat treatment, inoculation and fermentation. Initially, milk is heated to 95 °C for 1 h or for 15 min at 125 °C (Vedamuthu 2006) for the production of denatured proteins and release of peptides, which stimulates the growth of *L. acidophilus.* The milk is then cooled to 37 °C and kept for 3–4 h to allow spores present to germinate, followed by sterilization to destroy any vegetative cells and cooling to 37 °C. An active bulk culture of *L. acidophilus* is inoculated into the heat-treated milk at a rate of about 2–5 %, and is allowed to ferment until the pH drops to 5.5–6.0 or approximately 1 % of lactic acid is obtained (Surono and Hosono 2011). The number of viable *L. acidophilus* colonies increases to 2–3 × 10^9 CFU/mL during fermentation for 18–24 h., but this decreases with time, and the count may be reduced with extended incubation time. Co-culturing of *L. acidophilus* with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* (subs. *bulgaricus*) is often preferred. Finally, the product is rapidly cooled to less than 7 °C and then pumped into the packaging containers (Kosikowski and Mistry 1997; Vedamuthu 2006).

Table 3.3 Production and characteristics of acidophilus milk

Product	Starter organism	Fermentation	Additives	Specifications
Acidophilus milk	*Lactobacillus acidophilus*	At 37 °C until pH 5.5–6.0 (usually takes 18–20 h). Inoculation level 2–5 %	Enrichment with minerals and vitamins is possible	Distinctive tangy flavor and slightly thickened texture
Sweet acidophilus milk	*Lactobacillus acidophilus*	No fermentation is allowed. *L. acidophilus* is added to pasteurized milk at >5 °C	Enrichment with minerals and vitamins is possible	Sweet flavor, extended shelf life to 14 days (if freeze-dried, shelf life can be extended up to 28 days)
Acidophilin	*Lactobacillus acidophilus*, kefir yeasts	Fermentation is achieved at 35 °C	Whole or skimmed milk is fortified with skim milk powder, sucrose or cream	Acidophilin is used to treat colitis, enterocolitis, dysentery and other intestinal diseases. The product is sweeter than acidophilus-yeast milk
Diphilus milk	*Lactobacillus acidophilus*, *B. bifidum*	Fermentation is achieved at 37°C until pH 4.5–4.6.	N/A	Produced from cow's milk and has a specific taste and aroma. Used in therapy for intestinal disorders.
Acidophilus Bifidus milk	*Lactobacillus acidophilus*, *B. bifidum*	Fermentation is achieved at 37 °C until pH 4.5–4.6	Protein enrichment and fat standardization are common practices	Shelf life of the product is around 20 days, with an average number of probiotic bacteria of 10^8–10^9 CFU /mL
Bifighurt	*Bifidobacterium longum* (CKL 1969) or *Bifidobacterium longum* (DSM 2054)	Fermentation is achieved at 42 °C until pH 4.7 For human strains of probiotic bacteria Fermentation is set at 37 °C	N/A	Slimy texture with a characteristic slightly acidic flavor

(continued)

Table 3.3 (continued)

Product	Starter organism	Fermentation	Additives	Specifications
		Inoculation level of probiotic strains is around 6 %		
Bifidus milk	*Bifidobacterium bifidum* or *B. longum*	Inoculation level is 10 %. Fermentation is achieved at 37 °C until pH 4.5–4.6	Protein enrichment and fat standardization are common practices	Slightly acidic flavor and characteristic aroma with a lactic acid-to-acetic acid ratio of 2:3
Yakult	*Lactobacillus casei* Shirota	Fermentation is achieved at 37 °C until pH 4.5–4.6 (usually takes 16–18 h)	Total solids and sugar levels are adjusted to 3.7 and 14 % prior to heat treatment. It is common practice to add nature-identical flavors (e.g. tomato, celery, carrot)	The shelf life of the product is about 30 days. Regular consumption of this product has a positive effect on natural killer (NK) cell activity in middle-aged people
Yakult Miru-Miru	*Lactobacillus casei, Bifidobacterium bifidum* or *Bifidobacterium breve*, *Lactobacillus acidophilus*	Fermentation is achieved at 37 °C until pH 4.5–4.6	Addition of saccharides is a common practice	This product is broadly similar in composition to cow's milk
Acidophilus-yeast milk	*Lactobacillus acidophilus, Saccharomyces lactis*	Fermentation is achieved at 35 °C until 0.8 % lactic acid and 0.5 % ethanol levels are reached	N/A	Viscous and slightly acidic product with a yeasty taste

Modified from Özer and Kirmaci (2010)
N/A Not available

The production of bifidus milk, which contains *Bifidobacterium bifidum*, is similar to that of acidophilus milk. Bifidus milk often contains lactic and acetic acids in a ratio of 2:3, making it acidic in nature. The production of probiotic milk with both acidophilus and bifidum bacteria is also possible, and such milk has a characteristic aroma and is slightly acidic (Homayouni et al. 2012).

The protein content of fermented and non-fermented milk is similar, but higher amounts of free amino acids are present in acidophilus milk. It can also be enriched by the addition of calcium, iron and vitamins. In acidophilus milk products, lactose is hydrolyzed by β-galactosidase enzymes present in *Lactobacillus acidophilus*, which makes them consumable by lactose-intolerant individuals. However, due to the high acid content, their sour taste can reduce the market for these products. This has led to the development of sweet acidophilus milk. The bacteria used in probiotic milk products are viable for 14 days when stored at <10 °C after packaging. Freeze-dried cultures, however, have shown better results, with viability for 23 days at 4 °C reported for sweet acidophilus milk. Therefore, frozen products have gained much attention in the production of probiotic foods (Vedamuthu 2006).

3.2.1.2 Probiotic Cheese

Cheese is another dairy product which supports the survival of probiotic microbes, and is thus considered a good vehicle for transporting probiotics into the human body. This can be achieved either by producing cheese that favors the requirements of probiotic strains, or by developing appropriate probiotic strains to adapt to the cheese (Homayouni et al. 2012).

Both *L. acidophilus* and *B. bifidum* are common probiotics used in cheese products. Various cheese varieties such as cheddar (Dinakar and Mistry 1994; Gardiner et al. 1998), gouda (Gomes et al. 1995), cottage (Blanchette et al. 1996; O'Riordan and Fitzgerald 1998) and white-brined (Ghoddusi and Robinson 1996) cheeses have been assessed with these probiotic strains.

Production Process

Cheddar cheese has been found to be an efficient carrier of probiotics into the gastrointestinal tract. Certain microbial strains such as *Enterococcus faecium* (Gardiner et al. 1999) have shown better viability, stability and activity in cheddar cheeses than in other varieties of cheese. Gardiner et al. (1999) reported that when the probiotic culture was exposed to porcine gastric juice with a pH of 2, cheddar cheese showed a greater protective effect than yogurt. Cheddar cheese is manufactured under controlled bacteriological conditions to reduce contamination (McSweeney et al. 1994). The standard method of preparing cheddar cheese is initiated by curdling with the addition of starter and probiotic microbes followed by the rennet enzyme. Curds are cooked and milled at 39 °C and the pH is maintained at 6.1. Salt is added to a level of 2.8 % (wt/wt). The curds are then placed in the template molds, and pressure (200–400 kPa) is applied. The produced curd is then vacuum-packed. It is recommended that the packed cheese is stored at or below 8 °C in order to maintain the viability of the probiotic culture (Gardiner et al. 1998).

Research on the production of probiotic cheese has given rise to precautions and insights, which in turn have resulted in better products. It has been suggested that oxygen content and water activity of probiotic cheese must be evaluated before packaging (Dave and Shah 1997). Roy and Mainville (1997) reported that a high

survival rate and stability of probiotic microorganisms could be achieved by cooling the product. In addition, low temperature inhibits the interaction between the probiotic microorganism and cheese components. The subtle interaction of probiotic microbes in the cheese depends on the type and quantity of sugars available, degree of hydrolysis of milk proteins and lipids, availability of free amino acids and availability of short-chain fatty acids (Fox et al. 1996).

Antagonism between probiotic microbes and the starter organisms in cheese has also been reported. This is mainly caused by bacteriocins, peptides or proteins with antibiotic properties, which are considered limiting factors in production (Joseph et al. 1998). Such antagonism may also be caused by other constituents such as hydrogen peroxide, benzoic acid, biogenic amines and lactic acid. These effects also depend on whether the probiotics are added before or after fermentation. Interactions and metabolic activity may be reduced by lowering the temperature (<8 °C).

The final taste and flavor of the probiotic cheese is mainly affected by the proteolytic and lipolytic properties of the probiotic microbes (Kunji et al. 1996). Cheddar cheese is considered a potent vehicle for carrying these probiotic microbes to the human gastrointestinal tract, due to its dense solid structure and fat content, which helps to protect the probiotic bacteria. In addition, low pH creates a buffer environment around it and thus provides favorable conditions for the survival of the probiotic strains (Ross et al. 2002; Bergamini et al. 2005).

3.2.1.3 Probiotic Yogurt

Yogurt is the most popular probiotic consumer product, and is preferred for its nutritional value, health benefits and various therapeutic effects. Increased awareness of the importance of probiotics has made probiotic yogurt a common product in most grocery stores. Traditionally, yogurt has been made with *Lactobacillus bulgaricus* and *Streptococcus salivarius* as starter cultures. However, they are not able to survive the gastrointestinal tract environment and are thus not considered for use as probiotics in fermented products. Therefore, the addition of probiotic microbes (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) is preferred due to their value and viability.

Production Process

Probiotic yogurt production is similar to traditional yogurt manufacturing except for the inoculation of additional probiotic microbial cultures. Initially, starter microbial cultures (*L. bulgaricus* and *S. salivarius*) are added to the heat-treated homogenized milk with increased protein content (3.6–3.8 %) and incubated for 3.5 h at 45 °C or 9 h at 37 °C. The probiotic cultures are either added with the starter culture or after the first fermentation. The product is cooled to 4 °C before packaging. The viability of the probiotic microorganisms depends on several factors, including carbohydrate composition, fermentation medium, antagonism, culture conditions, dissolved oxygen (especially for *Bifidobacterium* spp.), incubation, and storage temperature and duration.

It has been reported that the viability of probiotic bacteria, especially *Bifidobacterium*, is reduced at refrigerated storage temperatures due to antagonistic effects (Dave and Shah 1997). One way to overcome this is to grow the *Bifidobacterium* spp. separately, wash out the free metabolites, and then transfer the cells to the probiotic yogurt (Homayouni et al. 2012). *Bifidobacterium* species are highly anaerobic in nature, and therefore, high dissolved oxygen content in milk is a critical constraint on their growth. To address this problem, a high-oxygen-utilizing bacteria, *S. thermophilus*, has been used along with *Bifidobacterium* species to maintain low levels of dissolved oxygen in yogurt. Yogurt must be stored at low temperatures for better viability of probiotic microbes.

3.2.2 Whey (Protein)-Based Probiotic Products

Whey, also referred to as a milk serum-based product, has gained much attention in recent years because of its high nutritional and low calorific value, thirst-quenching character and lower acidity than fruit juices. The nutritional value of whey basically depends on the milk. Some of the constituents in whey include lactose (70 %), proteins (beta-lactoglobulin, alpha-lactalbumin, serum albumin) and some minerals, along with certain vitamins (most of the B complex vitamins and some others) (Goyal and Gandhi 2008). Extensive research has focused on the health benefits of whey products. Weight gain and reduced incidence of diarrhea in pigs (Shilovskya 1983) and calves (Navetal et al. 1987) have been reported.

High lactose (sugar) content in whey supports the growth of probiotic microbes. Whey protects the microbes against the highly acidic environment of the gastrointestinal tract by increasing the level of pH in its immediate environment, and promotes the survival and viability of probiotic microorganisms. Therefore, whey is considered an efficient carrier for the transport of probiotics into the gut. In addition, whey provides a favorable medium for the survival of probiotic microbes during storage at low temperatures. Hernandez-Mendoza et al. (2007) studied changes in microbial count, pH values and titratable acidity during the storage of whey-based probiotic beverages. The authors reported no change in product parameters during storage for 30 days at 4 °C, except for slight acidification, but the beverage retained its acceptable flavor.

Lactobacillus and *Bifidobacterium* species are widely used probiotic microbes in whey-based products as well. Whey probiotic drinks containing *L. acidophilus* have shown preventive effects against diarrhea in children. The health-promoting probiotic microbes not only increase the flavor and texture of the whey-based products, but they also provide nutrition and various strain-specific health benefits (Katz 2001). One of the limitations of probiotic whey products is high transportation costs due to the volume occupied by liquid whey. This can be overcome, however, by concentrating the product through evaporation, reverse osmosis or ultrafiltration to occupy less volume but with the same relative composition.

In addition to probiotic microbes, a number of bioactive components with similar activity are being used in various functional food products. Some of the manufacturers of non-probiotic functional milk products are listed in Table 3.4 (**Advanced Non-food Applications of Probiotics**).

Probiotics have been shown to confer various health benefits to humans (Aureli et al. 2011), animals (Iannitti and Palmieri 2010) and plants (Song et al. 2012). However, it is necessary to ensure that processing conditions applied during probiotics production enable them to retain their activity and viability. Probiotics can be applied across a wide range of areas beyond food and beverages.

3.3 Applications of Probiotics

3.3.1 Medical Applications

Probiotics exert certain effects on various pathological diseases and gastrointestinal and extra-intestinal disorders, including the prevention or alleviation of symptoms of traveler's diarrhea and antibiotic-associated diarrhea, inflammatory bowel disease (Marteau et al. 2002) and lactose intolerance (de Vrese et al. 2001), and protection against intestinal infections (Reid et al. 2001) and irritable bowel syndrome. Though all probiotic microorganisms show various health benefits, there is no single organism capable of conferring all proposed benefits (Vasiljevic and Shah 2008). In addition to improved immune response in elderly people, medicinal properties observed in probiotics include improvements in patients suffering from rheumatoid arthritis and liver cirrhosis (Ibrahim et al. 2010), reduced prevalence of atopic eczema (Gueimonde et al. 2006), and reduced risk of colon cancer through inhibition of carcinogens and of bacteria capable of converting pro-carcinogens into carcinogens (Vasiljevic and Shah 2008).

Some researchers (Burns and Rowland 2000) have reported important medicinal characteristics of probiotics including anti-genotoxicity, anti-mutagenicity and anti-carcinogenicity. However, clinical trials show mixed results. Some studies suggest that certain probiotics may help in maintaining remission of ulcerative colitis and preventing relapse of Crohn's disease and recurrence of pouchitis (a complication of surgery to treat ulcerative colitis). These studies are still in the research phase, and further work is needed to prove strain-specific effects.

Probiotics have been suggested to be helpful in maintaining the microflora in the urogenital ecosystem. Lactobacilli play an important role in inhibiting the growth of pathogenic microbes by creating highly acidic environments, but this can be disturbed by various factors such as antibiotics, spermicides and birth control pills. Probiotic treatment may restore the microflora balance and help in curing some common female urogenital problems such as bacterial vaginosis, yeast infection and urinary tract infection (Harvard 2005).

Current research on probiotic applications in disease control has proven that some probiotic microbes (e.g. lactic acid bacteria) are helpful for delivering cytokines directly to the targeted sites in the host body (Behnsen et al. 2013).

3.3.2 Soil Fertility in Agriculture

Soil fertility plays a major role in agriculture, and is dependent on the bacteria and fungi in the soil. These microorganisms grow on organic matter and degrade into small molecules which can be taken up by plants through their roots. Good soil fertility can be achieved by using very low levels of synthetic herbs or pesticides and fertilizers, and therefore by using probiotic microorganisms. Nowadays, specific microorganisms are isolated from plant sources and cultured on a commercial scale, and are then used as bio fertilizers or biological control agents for plant diseases (Berg 2009). These microorganisms help to suppress plant pathogens and promote plant growth (Perrig et al. 2007; Saleem et al. 2007; Sheng et al. 2008; Compant et al. 2010).

3.3.3 Veterinary Applications

Recent studies have shown enormous health benefits of probiotics in animals. Animal feed with efficient probiotic microorganisms benefits animal health, maintains the microbial balance in the gut and aids in the digestion of food in the gastrointestinal tract. The use of probiotics in animal feed can enhance immunity, increase daily weight gain and feed efficiency in feedlot cattle, enhance milk production in cows, and improve health performance in calves (Seo et al. 2010) and chickens (Kalavathy et al. 2003). Probiotic microorganisms compete with the pathogenic bacteria in the gut for attachment to the mucosal wall and adjust to the immune response (Vine et al. 2004). They can aid the growth of non-pathogenic microbes and gram-positive bacteria in the gut by inhibiting pathogenic bacterial growth. Probiotic strain do this by producing hydrogen peroxide and volatile fatty acids (Jin et al. 2000). They are also efficient in stimulating the synthesis of B complex vitamins and improving the immune response in animals (Excelife 2006).

3.3.4 Aquaculture Enhancement

Probiotics are well known for their beneficial properties such as pathogen inhibition in aquatic organisms. In China alone, the market demand for commercial probiotics was reported at over 50,000 tons, with an approximate value of €50 million (Qi et al. 2009). Probiotics are used in the form of feed as growth promoters for

Table 3.4 Some commercial non-probiotic functional drinks

Country	Producer	Brand	Type of product	Bioactive components
Australia	PB food Australia	Heart plus	Low-fat milk	Fish oil
	Dairy farmers	Farmers best	Low-fat milk	Vegetable oil
	Parmalat	Physical	Milk	Calcium
		Calcium Plus	Milk	Calcium
Belgium	Danone	Zen	Fermented milk drink	Magnesium
Canada	Danone	Danacol	Low-fat dairy drink	Phytosterol
	Parmalat	Beatrice	Chocolate milk	
	Neilson	Dairy Oh	Fresh and chocolate milk	
	Lactantia Parmalat	Lactantia Nature Addition	Low-fat milk	Flax seed oil
	Natrel	Natrel Omega-3	Low-fat milk	Organic flax seed oil
		Natrel Calcium	Milk	Calcium
France	Candia	Candia with omega-3	Low-fat milk	
		Viva	Milk	Magnesium
	Lactalis	Magnesio	Milk	Magnesium
Finland	Valio	Evolus®	Fermented dairy fruit beverage	Bioactive peptides obtained by milk fermentation Val-Pro-Pro/Ile-Pro-Pro
	Ingman Dairy	Night-Time Milk	Low-fat milk	Melatonin
Ireland	Dawn Dairy	Dawn omega Milk	Low-fat milk (fresh)	Fish oil
Japan	Calpis	Ameal S	Cultured milk	Bioactive peptides
	Meiji-Milk	Meiji Love	Milk	Calcium and iron
	Meiji-Love	Fe-Milk	Milk	Iron
	Stolle Milk	Alpha	CPP- and IgG-rich milk	CPP and IgG
	Kyodo Milk Japan	*Lactobacillus casei* with lactoferrin	Probiotic fermented milk	*Lactobacillus casei*, lactoferrin

(continued)

Table 3.4 (continued)

Country	Producer	Brand	Type of product	Bioactive components
Malaysia	Nestle	Omega Plus	Low-fat milk (UHT)	Vegetable oil
New Zealand	Anchor	Vital	Low-fat milk	
Singapore	Nestle	Omega Plus	Low-fat milk (UHT)	Corn oil
Spain	Corporación Alimentaria Peñasanta S. A.	Natura Linea	Milk-fruit juice drink	Conjugated linoleic acid (Cognis Tonalin brand)
UK	Unilever	Flora pro•activ	Yogurt drink	Phytosterol
			Low-fat milk	
	Waitrose and Red Kite Farms	Slumber Bedtime Milk	Low-fat milk	Melatonin
	Dairy Crest	St. Ivel Advance	Fresh milk	
	Mc Neil Nutritionals	Benecol	Yogurt drink	Phytosterol

Compiled from Özer and Kirmaci (2010)

cultivated species in aquaculture. Colony formation of probiotic microbes in the gut of the aquatic species depends on factors such as body temperature, enzyme level, genetic resistance and water quality (Martínez Cruz et al. 2012). Microalgae such as *Chaetoceros* spp., which are considered central diatoms, have been used as carriers for probiotic microorganisms including *Vibrio alginolyticus* C7b and grown together to feed shrimp (Gomez-Gil et al. 2002). In 2003, Lara-Flores et al. (2003) reported that providing a probiotic *Streptococcus* strain in the diet of Nile tilapia (*Oreochromis niloticus*) led to a significant increase in crude protein, lipid content and weight (from 0.154 to 6.164 g) observed at 9 weeks of culture feeding. In addition to the growth-promoting factor, probiotics act as inhibitors of pathogens and control certain diseases in aquatic organisms. The pathogens *Aeromonas hydrophila* and *Vibrio alginolyticus* were inhibited using probiotic strains isolated from the gastrointestinal tract of clownfish (*Amphiprion percula*) (Vine et al. 2004). Because of their anti-pathogenic activity, probiotics are increasingly being used in place of antibiotics. Many importing countries have banned animal and aquaculture products produced with antibiotic supplementation in feed, as the use of antibiotics in feed has already been shown to lead to the development of antibiotic resistance in many food pathogens (EU Ban 2005; Gilchrist et al. 2007; Nunes et al. 2012; Burt 2014).

3.4 Probiotic Stability

The production of stable probiotics supplements possessing desirable organoleptic characteristics and capable of imparting prolonged health benefits to a targeted individual, while simultaneously retaining the viability of the incorporated strains, is one of the greatest technological challenges at present. Probiotic strains included in food supplements must tolerate various unfavorable conditions during industrial processes (manufacturing and storage) and must survive the harsh and competitive environment of the gastrointestinal tract and other environmental conditions. Many of the prevalent probiotics in the market are fastidious microorganisms which are nutritionally demanding and extremely sensitive to parameters such as ambient temperature, pH, oxygen content, water activity and the presence of other chemicals and microorganisms (Chávarri et al. 2012; Gueimonde and Sánchez 2012). A major hindrance in the production of probiotic supplements is the loss of viability of the probiotic strains during the industrial processing of the product. In order to circumvent this problem, a number of technological and microbiological approaches have been adopted by various manufacturing units (Anal and Singh 2007; Gueimonde and Sánchez 2012).

3.4.1 Technological Approach for Stability of Industrial Probiotics

For a probiotic product to be effective, it must maintain the viability of the strain during its manufacture, distribution and storage. An accepted benchmark is that a person should consume at least 100 g (containing at least 10^8 to 10^9 viable cells) of probiotic cultures in order to meet the minimum required effective concentration (at least 10^6 CFU/g) to show beneficial health effects (Kechagia et al. 2013; Mitropoulou et al. 2013). In addition, a probiotic culture should not reduce the desirable organoleptic quality of the product. The viability of the probiotic strains and the organoleptic properties of products containing such strains appear to be negatively affected during manufacturing and storage. For instance, the viability of *Bifidobacterium,* a probiotic bacterium, in food products such as yogurt preparations is significantly affected by various physiochemical factors including pH, concentration of lactic and acetic acids, hydrogen peroxide, dissolved oxygen content and low storage temperatures (Shah et al. 1995; Mitropoulou et al. 2013). Thus it is of the utmost importance to develop technology capable of preserving the viability of *Bifidobacterium* and other probiotics in the product in order to impart the desired health benefits to the consumer. Furthermore, the health benefits must be achieved in a cost-effective manner.

Immobilization of viable cells is an important technique for preserving the viability of probiotic bacteria, as it helps to protect the probiotic microbes from adverse environmental conditions including changes in pH, temperature and various harmful microbial attacks (Gbassi and Vandamme 2012; Heidebach et al. 2012; Mitropoulou et al. 2013). One may find the terms "immobilization", "entrapment" and "encapsulation" used interchangeably in most of the literature on the microencapsulation of probiotics (Gbassi and Vandamme 2012; Mitropoulou et al. 2013). Immobilization is defined as the process of attaching a cell or entrapping it within a suitable inert material (called a matrix), while encapsulation is the process of forming a continuous coating around an inner matrix that is wholly contained within the capsule wall as a core of encapsulated material (Gbassi and Vandamme 2012). Among immobilization techniques (covalent bonding, adsorption, entrapment and encapsulation), probiotic encapsulation technology (PET) has emerged within the past decade as an exciting and rapidly developing technology. Another efficient method used in the probiotics industry is microencapsulation, which is the process of coating or entrapping a useful core material. This method results in tiny capsules ranging in size from a few micrometers to a few millimeters (Heidebach et al. 2012). This increases the mouth feel of the product.

Selection of a matrix material is a crucial step in carrying out any sort of immobilization technique, and it becomes more sensitive when the product is made in the food industry for human consumption. The carrier used in immobilization of probiotics should be chemically, physically and biologically stable, mechanically robust, easily available and non-toxic, and should have easy handling requirements. Other factors to consider, depending upon its application, include physical characteristics (porosity, swelling, compression and mean particle behavior) and the possibility for microbial growth, biodegradability and solubility (Mitropoulou et al. 2013). An immobilization carrier (support) to be used in the food industry must meet stringent rules and regulations; few are considered to be industrially applicable. Generally, biopolymers and natural supports of food-grade purity are preferred, and materials containing non-digestible carbohydrates are being explored as potential carriers for use in the future (Mitropoulou et al. 2013). In addition to alginate, which is most commonly used as support for the encapsulation of probiotics, potential probiotic carriers include chitosan-coated alginate beads, apple pieces, pear pieces, wheat grains, oat pieces, whey proteins, carrageenan, gelatin, cellulose acetate phthalate and locust bean gum (Gbassi and Vandamme 2012; Cláudia et al. 2013; Mitropoulou et al. 2013). Encapsulation techniques used in the probiotics food industry include spray drying, spray cooling, fluidized-bed agglomeration and cooling, freeze and vacuum drying, emulsion-based techniques, and coacervation and extrusion. (Chávarri et al. 2012). However, the majority of microcapsules of probiotic strains for use in the food industry are generated by either extrusion or emulsion. The use of spray drying as an alternative encapsulation technique has also recently emerged (Heidebach et al. 2012).

3.4.1.1 Extruded Beads

In this technique, probiotics strains are mixed with an aqueous hydrocolloid solution and then extruded. Typically, a syringe is used that extrudes the gelling liquid in the form of droplets. One of the most common extrusion techniques using sodium alginate involves dropping the mixture of probiotic strains and sodium alginate solution into calcium chloride solution from an appropriate height using a syringe. The size of the encapsulating capsules depends upon the diameter of the orifice of the extruder, dropping height and the viscosity of the hydrocolloid–cell mixture. The size of beads produced with this method generally ranges from 0.5 to 3 mm (Gbassi and Vandamme 2012; Heidebach et al. 2012).

3.4.1.2 Emulsion Precipitation

In this technique for encapsulation of probiotic cells, a water-in-oil (W/O) emulsion of aqueous hydrocolloid–cell mixture (discontinuous phase) and vegetable oil (continuous phase) is formed using a small volume of discontinuous phase and larger volume of continuous phase. Once this emulsion is formed, the dispersed hydrocolloid–cell mixture is insolubilized to form small beads within the oil phase. In the case of the formation of alginate capsules, the microcapsules are hardened by slow addition of calcium chloride solution to the emulsion while stirring the mixture. The hardened droplets settle to the bottom of the reservoir. With this technique, capsules less than 100 μm in size can be generated. Emulsification produces oily or aqueous droplets known as capsules, while extrusion produces gelled droplets, called beads. In addition, capsules differ in size and shape, compared to uniformly shaped beads (Gbassi and Vandamme 2012; Heidebach et al. 2012).

3.4.1.3 Spray Drying

In order to enhance the longevity of probiotic microcapsules, they are usually dried after production. Freeze drying is a common method for drying the capsules produced by extrusion or emulsification. Spray drying has emerged as an alternative method for achieving capsule formation and drying in a single step. The efficacy of this method in terms of protecting probiotic cell concentrates from various adverse conditions was investigated by spray drying the probiotic cell mixtures using aqueous solution of different polymers including modified starch, gum arabic, gelatin, whey protein isolate, maltodextrin mixed with gum arabic, and ß-cyclodextrin mixed with gum arabic. However, one of the biggest drawbacks of this technique for microcapsule formation is that the microcapsules in most cases are water-soluble, which makes them unsuitable for use in aqueous food products and the need for further protection during gastrointestinal transit (Heidebach et al. 2012).

3.4.2 Biological Approaches for Producing Industrially Stable Probiotics

Most probiotic strains used in industrial production either have inherent resistance to severe conditions they are expected to face or are adapted to help them acquire such characteristics. The selection approach includes (i) the selection of naturally available strains with the desired properties, (ii) stress adaptation of the naturally occurring strains, and (iii) genetic manipulation of the desired probiotic strains to produce genetically modified organisms (GMOs). (Gueimonde and Sánchez 2012; Novik et al. 2014).

3.4.2.1 Screening for Naturally Resistant Strains

Probiotic strains show varied resistance against pH, temperature and oxygen conditions, which in turn affects the stability and shelf life of probiotic foods. The selection of naturally available strains with desired traits for the production of probiotic products is desirable during manufacturing. One phenomenon that affects the stability of commonly available probiotics products such as yogurts and fermented milks is "post-acidification" or continuous acid production by the starter cultures during storage. This problem can be overcome by selecting starter cultures in which post-acidification will not occur. Secondly, the product is often exposed to oxygen-abundant conditions during its manufacture and storage. The use of aerotolerant species such as *Bifidobacterium animalis* subsp. *lactis* is often preferred. Thirdly, strains capable of producing exopolysaccharides (EPS), which are considered to have better tolerance of various stresses, have been suggested for industrial applications (Gueimonde and Sánchez 2012).

3.4.2.2 Acclimatization of Naturally Occurring Probiotic Strains

Probiotic strains with desirable characteristics and better adaptation to various stressful conditions can be produced from naturally occurring wild-type strains. Three major approaches are presently employed for producing more robust, industrially desirable strain: adaptation by employing gradually increasing stress, mutagenic treatment and treatment with selective pressure (Gueimonde and Sánchez 2012).

(a) **Adaptation by employing gradual increasing stress**
In this method strains are subjected to increasing sub-lethal stress conditions before exposing them to harsh conditions. This method has been employed for enhancing the heat and acid tolerance of the microorganisms in order to produce more stable probiotic products.

(b) **Mutagenic treatment**
Treatment of probiotic strains with various mutagens such as UV light or chemicals has improved the stability of the products in terms of acid tolerance, sensory attributes and metabolic activity (Gueimonde and Sánchez 2012). For example, UV mutagenesis and subsequent incubation in acidic medium led to improved stability of *B. animalis* subsp. *lactis* in low-pH juice (Saarela et al. 2011). Similarly, high production of acetic acid by the *Bifidobacterium* species has been a major limiting factor for its inclusion as a probiotic in fermented dairy products, as it confers undesirable organoleptic properties to the final product. Hence, new probiotic strains of *Bifidobacterium animalis* subsp. *lactis* CECT 7953 capable of producing low amounts of acetic acid have been developed from wild strains (*Bifidobacterium animalis* subsp. *lactis)* by random UV mutagenesis. These new strains, with reduced capacity for producing acetic acid, are desirable for the production of fermented dairy products (Margolles and Sánchez 2012).

(c) **Selective pressure treatment**
Stress-resistant derivatives may be obtained by exposing sensitive strains to selective pressure (stress factor). For example, sensitive strains of *Lactobacilli* and *Bifidobacterium* exposed to selective pressure were found to produce derivative strains with improved resistance to acid, bile, heat and oxygen (Collado and Sanz 2006; Berger et al. 2010). Additionally, such microbial derivatives have been shown to have stable phenotypes and cross-resistance to other stresses. This is an important advantage from a commercial point of view (Gueimonde and Sánchez 2012).

3.4.2.3 Producing Genetically Engineered Strains

The use of genetic engineering for improving the stability of probiotic strains is another exciting alternative. However, food infused with genetically modified organisms (GMOs) is still unacceptable in many countries and among large groups of consumers. Genetic modification of probiotic strains commonly involves one of the following (Gueimonde and Sánchez 2012; Novik et al. 2014):

(a) **Homologous expression**
This involves modifying the expression level of the prevailing gene of the microbial strain. For example, overexpression of a chaperone in *Lactobacillus paracasei* was found to increase strain stability (Desmond et al. 2004; Gueimonde and Sánchez 2012).

(b) **Heterologous expression**
This involves introducing the desired gene from one microbe into the probiotic strain of interest. For example, heterologous expression of the betaine uptake system (BetL) of *Listeria* into *Lactobacillus salivarius* was found to increase tolerance to acid and high osmolar conditions (Gueimonde and Sánchez 2012; Novik et al. 2014).

3.5 Safety of Probiotics

Probiotics are generally considered safe for human consumption, as no significant negative effects have been reported. Many members of probiotic strains such as *Lactobacillus* have been consumed along with various dairy products. Among the probiotics, *Bifidobacterium* and members of the lactic acid bacteria (LAB) genera *Lactococcus* and *Lactobacillus* are commonly believed to be safe and given the status "generally recognized as safe" (GRAS), whereas other genera, including *Streptococcus* and *Enterococcus*, are considered to be opportunistic pathogens (Salminen et al. 1998; Snydman 2008). Despite the many controlled clinical trials on the use of probiotics that have demonstrated safe use, in some instances consumption of probiotics has been linked with three health issues: (i) occurrence of diseases such as bacteremia or endocarditis, (ii) various adverse immunogenic responses, both localized and generalized, and (iii) transfer of antibiotic resistance to the other pathogens in the gastrointestinal tract. There is no evidence from population-based studies, however, of any increased risk of bacteremia or endocarditis due to probiotics (Snydman 2008). For example, *Lactobacillus* GG, a bacterial strain commonly used in probiotic therapy, has been associated with bacteremia and liver abscess in patients with short gut syndrome. However, because of the relatively small number of such cases, and the positive safety evaluation results obtained for this probiotic, it can be generally considered as safe (Salminen et al. 2002; Snydman 2008). Some concerns have been raised that probiotics might produce undesirable metabolites and cause colon cancer or degrade the intestinal mucus, but these theories are not supported by sufficient epidemiologic or clinical evidence. These hypotheses were further contradicted by experimental data obtained from research carried out in an animal model, which showed anti-tumorigenic properties of probiotics in colon cancer (Goldin et al. 1996; Snydman 2008). Other studies performed in gnotobiotic rats have shown no evidence of degradation of the intestinal mucus by probiotic bacteria (Berg 1980; Ishibashi and Yamazaki 2001; Snydman 2008). Lactic acid bacteria are intrinsically resistant to antibiotics, and the transfer of antibiotic resistance to harmful pathogens in the gastrointestinal tract has been raised as a concern for their use. In most cases, however, this resistance is not considered to be transmissible. Furthermore, these probiotics have shown sensitivity to many antibiotics in clinical use. Thus, even in cases where patients may develop lactic acid bacteria-associated opportunistic infections, they can be treated by conventional antibiotic therapy. However, transmissible enterococcal resistance against glycopeptide antibiotics (vancomycin and teicoplanin) should not be ignored, as vancomycin is one of the last remaining effective antibiotics for treating certain multidrug-resistant pathogens (Salminen et al. 1998). Additionally, many new species and more specific strains of probiotics are being isolated and characterized for probiotic use. These newly identified strains should be carefully assessed, and rigorous clinical trials and evaluation should be conducted on a case-by-case basis before their incorporation into food products. In the case of selecting novel strains, species and genera for probiotic use, current

safety assessment procedures described in the European Union (EU) novel foods directive must be strictly adhered to among EU member countries (Conzelmann 1997; Salminen et al. 1998). Although the overall safety record held by probiotics is excellent, they should be used with caution in certain patient groups, particularly in neonates born prematurely or immunocompromised individuals (Hickson 2011; Marchand 2012). It should also be noted that the efficacy of probiotics is both strain- and disease-specific, and they should be given in adequate amounts. Furthermore, the properties of probiotics vary among species and can be strain-specific. Individual strains can possess characteristics such as resistance to gastric acid and bile, the ability to colonize the mucosa, and antimicrobial activity (Jacobsen et al. 1999; Hickson 2011). Hence, it would not be wise to generalize the effects of one probiotic species or strain to others without confirmation in separate studies. For instance, *L. rhamnosus* GG is a specific bacterial probiotic strain (the nomenclature includes genus, species and strain) which is capable of preventing antibiotic-associated diarrhea (AAD), whereas other strains of *L. rhamnosus* may not have this effect. On the other hand, some species in the *Lactobacillus* genus may not act as probiotics (McFarland 2006; Hickson 2011).

3.6 Technological Hurdles

Probiotic encapsulation technology (PET) is one of the most acclaimed technologies for protecting the probiotic strains and ensuring the stability of the products against various adverse effects (Chávarri et al. 2012; Gbassi and Vandamme 2012). There has been a tremendous improvement in the use of this technology for the development of stable, high-quality food products that retain their organoleptic qualities. It has been used for the production of dairy-based probiotic products such as yogurt, milk and cheese, and has been extended to non-dairy products such as fruit juices, cookies and chocolates. Despite these improvements, however, there are still many technological hurdles that PET must overcome before it can be considered a full-fledged technology. These include the development of equipment to produce small, uniform capsules or beads, the selection of non-toxic encapsulation materials, development of capsules or beads compatible with the pH of the human digestive tract, detailed in vitro and in vivo studies of the effects of encapsulation on the safety of probiotic strains in humans, and assessing the cost of microencapsulation (Vidhyalakshmi et al. 2009; Rokka and Rantamäki 2010; Gbassi and Vandamme 2012).

Emulsification and extrusion are two of the most common PET procedures. The presence of residual oil in the encapsulated material during the production of probiotic capsules via emulsification may not be suitable for the development of low-fat dairy products, as this can cause a deterioration in texture and organoleptic characteristics. Furthermore, it is arguable that emulsifiers, surfactants and the residual oil used in the emulsification process may be toxic to probiotic cells and may interact with food components. Therefore, the development of microcapsules

using only aqueous gelling, without the use of oils, emulsifiers and surfactants, is of the utmost importance. Extrusion faces the challenges of scaling up dairy production for large quantities of beads (Gbassi and Vandamme 2012).

Another challenge is in determining the physicochemical characteristics of encapsulation materials in order to predict their disintegration or dissolution mechanisms under varying conditions of pH and salinity and their interaction with probiotic cells and other components present in the gut. In vitro studies aimed at delivering viable strains of probiotics to consumers should be undertaken through simulation using simple and reproducible gastrointestinal tract models (Vidhyalakshmi et al. 2009; Chávarri et al. 2012; Gbassi and Vandamme 2012).

PET makes use of encapsulation materials such as natural polymers and milk proteins, which are expensive, and also uses various raw materials such as oils and emulsifiers for the formation of capsules. These all add to the cost of manufacturing, making probiotics-incorporated supplements an expensive product. Thus a major challenge is in reducing the costs involved in PET (Chávarri et al. 2012; Gbassi and Vandamme 2012).

The production of hydrocolloid-based microcapsules is a common practice for the encapsulation of probiotic strains in the food industry, and it involves the use of a high-shear process (due to the highly viscous nature of the hydrocolloids) during emulsification. In some probiotic strains, this reduces the encapsulation yield (EY), a combined parameter that describes the survival of the cells and the efficacy of entrapment during encapsulation procedures (Capela et al. 2007; Ding and Shah 2009; Heidebach et al. 2012). An alternative method is protein-based microencapsulation. However, production of protein-based microcapsules sometimes requires slight modifications to existing methods or even the establishment of novel encapsulation techniques. In a number of cases, such modification have reduced the encapsulation yield (Picot and Lacroix 2004; Annan et al. 2008; Heidebach et al. 2012). Similarly, the use of lipid-based microcapsules for the protection of the probiotic strains lacks sufficient scientific evidence of its effectiveness and requires more research (Heidebach et al. 2012).

3.7 Present and Future of Probiotics

The present probiotics market is growing at a good pace. Based upon a market report from Transparency Market Research, global probiotics demand was valued at $27.9 billion in 2011, and is expected to reach $44.9 billion by 2018, representing a 6.8 % compound annual growth rate (CAGR) from 2013 to 2018 (Chr. Hansen 2013; Nutraceuticals world 2013). Demand in Asia-Pacific and Europe has dominated the global market, with Asia-Pacific expected to be the fastest-growing market in the future (Nutraceuticals world 2013).

Most probiotic supplements present in the market are dairy-based products. However, non-dairy beverages are expected to grow rapidly. Bernat et al. (2014) recently explored the production of fermented almond milk infused with probiotics

and successfully optimized the process to ensure reduced fermentation time and production of a stable product that retained the viable strains throughout manufacturing, storage and in vitro digestion (Song et al. 2012; Bernat et al. 2014).

Disruption of gut microbiota has been linked to chronic diseases such as autoimmune disorders, colon cancers, gastric ulcers, obesity, type 2 diabetes and cardiovascular disease, and the use of probiotics has been reported to mitigate or completely cure these diseases. There is also considerable evidence of the beneficial effects of probiotics for the treatment of pouchitis, inflammatory bowel disease, traveler's diarrhea, allergy, antibiotic-associated diarrhea and clostridium difficile infection (Aureli et al. 2011; Hickson 2011; Adam et al. 2012; Marchand 2012; Jz et al. 2013; Kurzweil 2014; Chen et al. 2014).

In addition to their use in maintaining digestive health, probiotics have also been explored for their use in the human immunodeficiency virus (HIV) community. Andrieu et al. (2014) reported the successful use of a vaccine consisting of inactivated simian immunodeficiency virus (SIV)mac239 particles, together with a living probiotics adjuvant (either the Calmette–Guérin bacillus, *L. plantarum* or *L. rhamnosus*), for protection against simian immunodeficiency virus (SIV) in monkeys, which is equivalent to HIV. This opens up the possibility of using probiotics for the treatment of HIV in the future (Andrieu et al. 2014). Advances in molecular biology techniques have enabled a much clearer understanding of the gut microbiota, and research is thus being focused on the production of symbiotic products (combination of prebiotics and probiotics). Since there can be variations in the types of resident bacterial groups among individuals depending upon geographical region, ethnicity, age group and dietary habits, it is crucial to undertake further studies of the gut microbiota based on these parameters. It will be necessary to develop indigenous probiotic strains based upon the target population to gain enhanced health benefits.

From an industrial perspective, probiotic products should be available in different dosage forms and customized solutions. It is also necessary to determine the mode of action of specific probiotic effects. However, due to conflicting results obtained from various clinical studies, health professionals must exercise caution in establishing the efficacy of probiotic strains or formulations in terms of specific health claims. Many of the observed effects of the use of probiotics have been strain-specific, and thus conclusions should not be extrapolated. The benefit versus potential risk of each strain must be considered, especially for their use in immunocompromised individuals or persons suffering from multiple chronic diseases. Probiotic products presently available in the market commonly contain *Lactobacillus, Bifidobacterium* and *Saccharomyces boulardii*, which represent only a fraction of microbiota. Organisms such as *Bacteroides*, which comprise the major population of gut microbiota, should be studied for their potential broader health benefits.

One of the biggest challenges faced by probiotic producers is retaining the viability of the probiotics throughout the manufacturing, storage and delivery process. To overcome this challenge, microencapsulation techniques have been employed. This concept has been extended to the production of nano-capsules

(nanotechnology) of the viable cells. Microencapsulation of different probiotic strains with alginates has been shown to improve the survival rate of those strains under highly acidic conditions (pH 2.0) and high bile salt concentrations and to improve heat tolerance compared to the free cells, thus making them more commercially attractive (Ding and Shah 2007; Song et al. 2012). In addition to alginates, microencapsulation using gelatin or vegetable gums has been carried out to protect acid-sensitive *Bifidobacterium* and *Lactobacillus* (Song et al. 2012). Similarly, desirable probiotics can be nano-encapsulated for specific delivery of those bacteria to certain parts of the gastrointestinal tract, where they will interact with specific receptors (Sekhon 2010). However, as discussed above, microencapsulation still needs to be improved, and the adoption of nanotechnology within the food industry for encapsulating live bacterial cells is still a new concept. The latter method should be used with caution, as very little is known about its impact on environmental and human health. Presently, no regulations exist that specifically control or limit the production of nano-sized particles, and this is mainly due to a lack of unbiased knowledge regarding the risks. Detailed studies of the ingredients, more options of media for industrial use, and product and process re-engineering are needed to make probiotics products more palatable and to increase market demand (Song et al. 2012; Grover et al. 2012).

3.8 Conclusions

The use of probiotics in the food industry is increasing, and is predicted to have a much larger market share in the future. Their health benefits have been studied extensively, but considerable research is still ongoing to determine their efficacy, safety and precise dosage. The use of probiotics is not limited to improvements in human health; it has also been extended to veterinary, agricultural and fishery sciences. Studies have also explored the use of probiotics for the treatment of dreadful diseases such as HIV and for their ability to treat chronic diseases such as type 2 diabetes, obesity and colon cancer. The industrial use of probiotics is limited mainly by the fact that the viability of the probiotics strains is reduced during the various manufacturing processes, storage and delivery to the consumer. Tremendous improvement has been made in encapsulation technologies, primarily extrusion and emulsion methods, which has resulted in more stable products and has helped preserve the viability of the probiotic cultures in the product. However, this technology still needs improvement, and alternative methods are being explored in order to make the manufacturing process more economical and the product more palatable.

Recent developments in molecular biology have helped to deepen our understanding of gut microbiota, and it is now known that more than 10,000 different bacteria are present in the gut. However, apart from common probiotics such as *Lactobacillus, Bifidobacterium* and *Saccharomyces boulardii,* research should be further directed towards the study of organisms such as *Bacteroides*, which constitute the predominant

flora in the gut, and which would further broaden the health application of probiotics. As there is considerable diversity of human gut microbiota by geographic region, ethnicity, age group, population and dietary habits, the choice of indigenous strains for target populations may bring greater benefit to the consumer.

The development of extensive sequencing methods has enabled metagenomic studies on the human gut microbiome to be carried out (Gueimonde and Collado 2012). With our knowledge of gut microbiota composition and activity, diseases related to microbiota aberrations in the gut will be identifiable, and it will be possible to develop probiotics that can overcome the effects of these aberrations. This will expand the scope of probiotics use for more specific health issues in the future.

References

Activia Delicious Probiotic Yogurt. http://www.danone.ca/en/products/activia. Accessed 8 Jan 2015

Activity HN, Takeda K, Okumura K (2007) Effects of a Fermented Milk Drink Containing Lactobacillus casei Strain Shirota. 791–793

Adam JK, Odhav B, Babu KS (2012) Probiotics: recent understandings and biomedical applications. 6:1–14

Agostini S, Goubern M, Tondereau V et al (2012) A marketed fermented dairy product containing Bifidobacterium lactis CNCM I-2494 suppresses gut hypersensitivity and colonic barrier disruption induced by acute stress in rats. Neurogastroenterol Motil 24:376–e172. doi:10.1111/j.1365-2982.2011.01865.x

Akin M (2005) Effects of inulin and different sugar levels on viability of probiotic bacteria and the physical and sensory characteristics of probiotic fermented ice-cream. Milchwissenschaft 60:297–301

Akin MB, Akin MS, Kırmacı Z (2007) Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice-cream. Food Chem 104:93–99. doi:10.1016/j.foodchem.2006.11.030

Anal AK, Singh H (2007) Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends Food Sci Technol 18:240–251. doi:10.1016/j.tifs.2007.01.004

Andrieu J-M, Chen S, Lai C et al (2014) Mucosal SIV vaccines comprising inactivated virus particles and bacterial adjuvants induce CD8(+) T-regulatory cells that suppress SIV-positive CD4(+) T-cell activation and prevent SIV infection in the macaque model. Front Immunol 5:297. doi:10.3389/fimmu.2014.00297

Annan NT, Borza aD, Hansen LT (2008) Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic Bifidobacterium adolescentis 15703T during exposure to simulated gastro-intestinal conditions. Food Res Int 41:184–193. doi:10.1016/j.foodres.2007.11.001

Aureli P, Capurso L, Castellazzi AM et al (2011) Probiotics and health: an evidence-based review. Pharmacol Res 63:366–376. doi:10.1016/j.phrs.2011.02.006

Bais HP, Weir TL, Perry LG et al (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266. doi:10.1146/annurev.arplant.57.032905.105159

BC Diary (2015) The probiotic effects of lactic acid bacteria. http://bcdairy.ca/milk/articles/the-probiotic-effects-of-lactic-acid-bacteria/. Accessed 13 Mar 2015

Behnsen J, Deriu E, Sassone-Corsi M, Raffatellu M (2013) Probiotics: properties, examples, and specific applications. Cold Spring Harb Perspect Med 3:1–16. doi:10.1101/cshperspect.a010074

Berg G (2009) Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18. doi:10.1007/s00253-009-2092-7

Berg RD (1980) Inhibition of escherichia coli translocation from the gastrointestinal tract by normal cecal flora in gnotobiotic or antibiotic-decontamiinated mice. 29:1073–1081

Bergamini CV, Hynes ER, Quiberoni A et al (2005) Probiotic bacteria as adjunct starters: influence of the addition methodology on their survival in a semi-hard Argentinean cheese. Food Res Int 38:597–604. doi:10.1016/j.foodres.2004.11.013

Berger B, Moine D, Mansourian R, Arigoni F (2010) HspR mutations are naturally selected in Bifidobacterium longum when successive heat shock treatments are applied. J Bacteriol 192:256–263. doi:10.1128/JB.01147-09

Bernardeau M, Gueguen M, Smith DGE et al (2009) In vitro antagonistic activities of Lactobacillus spp. against Brachyspira hyodysenteriae and Brachyspira pilosicoli. Vet Microbiol 138:184–190. doi:10.1016/j.vetmic.2009.03.020

Bernat N, Cháfer M, Chiralt A, González-Martínez C (2014) Development of a non-dairy probiotic fermented product based on almond milk and inulin. Food Sci Technol Int. doi:10.1177/1082013214543705

BioGaia BioGaia Gastrus. http://www.biogaia.com/product/biogaia-gastrus. Accessed 13 Mar 2015

Blanchette L, Roy D, Bélanger G, Gauthier SF (1996) Production of cottage cheese using dressing fermented by Bifidobaceria. J Dairy Sci 79:8–15. doi:10.3168/jds.S0022-0302(96)76327-0

Burns AJ, Rowland IR (2000) Anti-carcinogenicity of probiotics and prebiotics. Curr Issues Intest Microbiol 1:13–24

Burt R (2014) Probiotics future in beef production looks promising. Beef Mag

Capela P, Hay TKC, Shah NP (2007) Effect of homogenisation on bead size and survival of encapsulated probiotic bacteria. Food Res Int 40:1261–1269. doi:10.1016/j.foodres.2007.08.006

Castex M, Lemaire P, Wabete N, Chim L (2009) Effect of dietary probiotic Pediococcus acidilactici on antioxidant defences and oxidative stress status of shrimp Litopenaeus stylirostris. Aquaculture 294:306–313. doi:10.1016/j.aquaculture.2009.06.016

Champagne CP, Møllgaard H (2008) Handbook of fermented functional foods, 2nd edn. In: Farnworth ER (ed) Handb. fermented Funct. foods, 2nd Editio. CRC Press, Boca Raton, p 600

Chávarri M, Marañón I, Villarán MC (2012) Encapsulation technology to protect probiotic bacteria

Chen Z, Guo L, Zhang Y et al (2014) Incorporation of therapeutically modified bacteria into gut microbiota inhibits obesity. J Clin Invest 124:3391–3406. doi:10.1172/JCI72517

Chr. Hansen (2013) Expanding production facilities for probiotics. http://www.chr-hansen.com/news-media/singlenews/expanding-production-facilities-for-probiotics.html

Cláudia S, Martins S, Martins CM et al (2013) Immobilization of microbial cells: a promising tool for treatment of toxic pollutants in industrial wastewater. 12:4412–4418. doi:10.5897/AJB12.2677

Collado MC, Sanz Y (2006) Method for direct selection of potentially probiotic Bifidobacterium strains from human feces based on their acid-adaptation ability. J Microbiol Methods 66:560–563. doi:10.1016/j.mimet.2006.01.007

Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669–678. doi:10.1016/j.soilbio.2009.11.024

Conzelmann C (1997) Foods: consequences for the food industry. 81

Culturelle® (2013) Culturelle® digestive health capsules. http://www.culturelleprobiotic.ca/digestive-health-probiotic-capsules. Accessed 13 Mar 2015

Danisco Winning probiotic solutions for poultry, swine and ruminants. http://animalnutrition.dupont.com/productsservices/feed-probiotic-solutions/. Accessed 8 Jan 2015

Danon Actimel Actimel Original Yoghurt. http://www.actimel.co.uk/fabulous-flavours/original/. Accessed 13 Mar 2015

Dave RI, Shah NP (1997) Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. Int Dairy J 7:31–41. doi:10.1016/S0958-6946(96)00046-5

De Vrese M, Stegelmann A, Richter B et al (2001) Probiotics–compensation for lactase insufficiency. Am J Clin Nutr 73:421S–429S

Desmond C, Fitzgerald GF, Stanton C, Ross RP (2004) Improved stress tolerance of GroESL-overproducing Lactococcus lactis and probiotic Lactobacillus paracasei NFBC 338. Appl Environ Microbiol 70:5929–5936. doi:10.1128/AEM.70.10.5929-5936.2004

Dinakar P, Mistry VV (1994) Growth and viability of Bifidobacterium bifidum in cheddar cheese. J Dairy Sci 77:2854–2864. doi:10.3168/jds.S0022-0302(94)77225-8

Ding WK, Shah NP (2009) Effect of homogenization techniques on reducing the size of microcapsules and the survival of probiotic bacteria therein. J Food Sci 74:M231–M236. doi:10.1111/j.1750-3841.2009.01195.x

Ding WK, Shah NP (2007) Acid, bile, and heat tolerance of free and microencapsulated probiotic bacteria. J Food Sci 72:M446–M450. doi:10.1111/j.1750-3841.2007.00565.x

Enviva (2013) Enviva® MPI- the complementary feed for promoting gut health. http://animalnutrition.dupont.com/resource-library/result-list/resource/show/List/envivaR-mpi/. Accessed 8 Jan 2015

EU Ban (2005) Ban on antibiotics as growth promoters in animal feed enters into effect. http://europa.eu/rapid/press-release_IP-05-1687_en.htm. Accessed 18 Mar 2015

Excelife (2006) B-imune multivitamin. http://www.webnificent.com/sample-sites/Excelife/multivitamin.htm

Florastor (2009) Florastor® a probiotic.org expert product review. http://www.probiotic.org/florastor.htm. Accessed 13 Mar 2015

Fox PF, Wallace JM, Morgan S et al (1996) Acceleration of cheese ripening. Antonie Van Leeuwenhoek 70:271–297. doi:10.1007/BF00395937

Francavilla R, Maurogiovanni G, Ierardi E, Cavallo L (2014) Lactobacillus reuteri strain combination in helicobacter pylori infection. J Clin Gastroenterol 48:407–413

Fuller R (1989) Probiotics in man and animals. J Appl Bacteriol 66:365–378

Gardiner G, Ross RP, Collins JK et al (1998) Development of a probiotic cheddar cheese containing human-derived Lactobacillus paracasei strains. Appl Environ Microbiol 64:2192–2199

Gardiner G, Stanton C, Lynch PB et al (1999) Evaluation of cheddar cheese as a food carrier for delivery of a probiotic strain to the gastrointestinal tract. J Dairy Sci 82:1379–1387. doi:10.3168/jds.S0022-0302(99)75363-4

Gbassi GK, Vandamme T (2012) Probiotic encapsulation technology: from microencapsulation to release into the gut. Pharmaceutics 4:149–163. doi:10.3390/pharmaceutics4010149

Ghoddusi HB, Robinson RK (1996) The test of time. Diary Ind Int 61:25–28

Gilchrist MJ, Greko C, Wallinga DB et al (2007) The potential role of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance. Environ Health Perspect 115:313–316. doi:10.1289/ehp.8837

Goldin BR, Gualtieri LJ, Moore RP (1996) The effect of Lactobacillus GG on the initiation and promotion of DMH-induced intestinal tumors in the rat. Nutr Cancer 25:197–204. doi:10.1080/01635589609514442

Gomes AMP, Malcata FX, Klaver FAM, Grande HJ (1995) Incorporation and survival of Bifidobacterium sp. strain Bo and Lactobacillus acidophilus strain Ki in a cheese product

Gomez-Gil B, Roque A, Velasco-Blanco G (2002) Culture of Vibrio alginolyticus C7b, a potential probiotic bacterium, with the microalga Chaetoceros muelleri. Aquaculture 211:43–48. doi:10.1016/S0044-8486(02)00004-2

Goyal N, Gandhi D (2008) Whey, a carrier of probiotics against diarrhoea. http://www.dairyscience.info/index.php/probiotics/110-whey-probiotics.html?showall=&start=2. Accessed 18 Mar 2015

Grover S, Rashmi HM, Srivastava AK, Batish VK (2012) Probiotics for human health -new innovations and emerging trends. Gut Pathog 4:15. doi:10.1186/1757-4749-4-15

Gueimonde M, Collado MC (2012) Metagenomics and probiotics. Clin Microbiol Infect 18 (Suppl):32–34. doi:10.1111/j.1469-0691.2012.03873.x

Gueimonde M, Kalliomäki M, Isolauri E, Salminen S (2006) Probiotic intervention in neonates–will permanent colonization ensue? J Pediatr Gastroenterol Nutr 42:604–606. doi:10.1097/01.mpg.0000221897.45910.d3

Gueimonde M, Sánchez B (2012) Enhancing probiotic stability in industrial processes. Microb Ecol Health Dis. doi:10.3402/mehd.v23i0.18562

Guybro Guybro chemical- Probiotics Products. http://www.indiamart.com/guybrochemical/probiotics-products.html. Accessed 8 Jan 2015

Guyonnet D, Chassany O, Ducrotte P et al (2007) Effect of a fermented milk containing Bifidobacterium animalis DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults in primary care: a multicentre, randomized, double-blind, controlled trial. Aliment Pharmacol Ther 26:475–486. doi:10.1111/j.1365-2036.2007.03362.x

Halliwell DE (2002) Hands up for probiotics! The mechanism for health benefits is unclear, but interest. World Food Ingredients 46–50

Harvard (2005) Health benefits of taking probiotics. http://www.health.harvard.edu/vitamins-and-supplements/health-benefits-of-taking-probiotics. Accessed 14 Mar 2015

Heidebach T, Först P, Kulozik U (2012) Microencapsulation of probiotic cells for food applications. Crit Rev Food Sci Nutr 52:291–311. doi:10.1080/10408398.2010.499801

Hernandez-Mendoza A, Robles VJ, Angulo JO et al (2007) Preparation of a whey-based probiotic product with Lactobacillus reuteri and Bifidobacterium bifidum. Food Technol Biotechnol 45:27–31

Hickson M (2011) Probiotics in the prevention of antibiotic-associated diarrhoea and Clostridium difficile infection. Therap Adv Gastroenterol 4:185–197. doi:10.1177/1756283X11399115

Hickson M, D'Souza AL, Muthu N et al (2007) Use of probiotic Lactobacillus preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. BMJ 335:80. doi:10.1136/bmj.39231.599815.55

Hilliam M (2004) Healthier dairy. World Food Ingredients 52–54

Homayouni A, Alizadeh M, Alikhah H, Zijah V (2012) Functional dairy probiotic food development: trends, concepts and products. doi:10.5772/3444

Iannitti T, Palmieri B (2010) Therapeutical use of probiotic formulations in clinical practice. Clin Nutr 29:701–725. doi:10.1016/j.clnu.2010.05.004

Ibrahim F, Ruvio S, Granlund L et al (2010) Probiotics and immunosenescence: cheese as a carrier. FEMS Immunol Med Microbiol 59:53–59. doi:10.1111/j.1574-695X.2010.00658.x

Ishibashi N, Yamazaki S (2001) Probiotics and safety. Am J Clin Nutr 73:465S–470

Jacobsen CN, Nielsen VR, Hayford AE, Møller PL (1999) Screening of probiotic activities of forty-seven strains of Lactobacillus spp. by in vitro techniques and evaluation of the colonization ability of five selected strains in humans. 65:4949–4956

Jin LZ, Marquardt RR, Zhao X (2000) A strain of Enterococcus faecium (18C23) inhibits adhesion of enterotoxigenic Escherichia coli K88 to porcine small intestine mucus. Appl Environ Microbiol 66:4200–4204

Joseph P, Dave R, Shah N (1998) Antagonism between yoghurt bacteria and probiotic bacteria isolated from commercial starter cultures, commercial yoghurts and a probiotic capsule. Food Aust 50:20–23

Jz G, Ssy M, Jd S et al (2013) Probiotics for the prevention of Clostridium difficile- associated diarrhea in adults and children (Review)

Kalavathy R, Abdullah N, Jalaludin S, Ho YW (2003) Effects of Lactobacillus cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. Br Poult Sci 44:139–144. doi:10.1080/0007166031000085445

Katz F (2001) Active cultures add function to yogurt and other foods. Food Technol 55:46–49

Kechagia M, Basoulis D, Konstantopoulou S et al (2013) Health benefits of probiotics: a review. ISRN Nutr 1–7

KeLATOX Life Start 2, Natren, Infant formula. http://www.kelatox.com/Life Start 2. Accessed 8 Jan 2015

Kosikowski FV, Mistry VV (1997) Fermented milks. Cheese Fermented Milk Foods, FV Ksikows. Westport, pp 57–74

Kosin B, Rakshit SK (2006) Microbial and processing criteria for production of probiotics: a review. Food Technol Biotechnol 44:371–379

Kunji ER, Mierau I, Hagting A et al (1996) The proteolytic systems of lactic acid bacteria. Antonie Van Leeuwenhoek 70:187–221

Kurzweil (2014) Study suggests probiotics could prevent obesity and insulin resistance. http://www.kurzweilai.net/study-suggests-probiotics-could-prevent-obesity-and-insulin-resistance

Lallemand BACTOCELL® Aquaculture—the dietary probiotic to secure fish and shrimp performance. http://www.lallemandanimalnutrition.com/products/bactocell-aquaculture/. Accessed 8 Jan 2015

Lara-Flores M, Olvera-Novoa MA, Guzmán-Méndez BE, López-Madrid W (2003) Use of the bacteria Streptococcus faecium and Lactobacillus acidophilus, and the yeast Saccharomyces cerevisiae as growth promoters in Nile tilapia (Oreochromis niloticus). Aquaculture 216:193–201. doi:10.1016/S0044-8486(02)00277-6

Lee K-W, Li G, Lillehoj HS et al (2011) Bacillus subtilis-based direct-fed microbials augment macrophage function in broiler chickens. Res Vet Sci 91:e87–e91. doi:10.1016/j.rvsc.2011.01.018

Lifeproducts 10-G Microbial feed additive for diary cattle

Marchand V (2012) Using probiotics in the paediatric. Can Paediatr Soc 17:1–17

Margolles A, Sánchez B (2012) Selection of a Bifidobacterium animalis subsp. lactis strain with a decreased ability to produce acetic acid. Appl Environ Microbiol 78:3338–3342. doi:10.1128/AEM.00129-12

Marteau P, Seksik P, Jian R (2002) Probiotics and intestinal health effects: a clinical perspective. Br J Nutr 88(Suppl 1):S51–S57. doi:10.1079/BJN2002629

Martínez Cruz P, Ibáñez AL, Monroy Hermosillo Oa, Ramírez Saad HC (2012) Use of probiotics in aquaculture. ISRN Microbiol 2012:916845. doi:10.5402/2012/916845

McFarland LV (2006) Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease. Am J Gastroenterol 101:812–822. doi:10.1111/j.1572-0241.2006.00465.x

McSweeney PLH, Walsh EM, Fox PF et al (1994) A procedure for the manufacture of cheddar cheese under controlled bacteriological conditions and the effect of adjunct Lactobacilli on cheese quality. Irish J Agric Food Res 33:183–192

Mitropoulou G, Nedovic V, Goyal A, Kourkoutas Y (2013) Immobilization technologies in probiotic food production. J Nutr Metab 2013:716861. doi:10.1155/2013/716861

Navetal H, Remsey C, Demigne C, Ascher F (1987) Supplemented whey for treating chalky diarrhoea of calves. Dairy Sci Abstr 49:3707

Novik G, Sidarenka A, Kiseleva G et al (2014) Probiotics. In: Brar S, Dhillon GS, Fernandes M (eds) Biotransformation waste biomass into high value biochem. XV. Springer Science & Business Media, New York, NY, pp 187–235

Nunes RV, Scherer C, Pozza PC et al (2012) Use of probiotics to replace antibiotics for broilers. Rev Bras Zootec 41:2219–2224. doi:10.1590/S1516-35982012001000012

Nutraceuticals world (2013) Global Probiotics Market Expected to Reach $44.9 Billion by 2018. http://www.nutraceuticalsworld.com/contents/view_breaking-news/2013-01-11/global-probiotics-market-expected-to-reach-449-billion-by-2018/

O'Riordan K, Fitzgerald GF (1998) Evaluation of bifidobacteria for the production of antimicrobial compounds and assessment of performance in cottage cheese at refrigeration temperature. J Appl Microbiol 85:103–114

Özer BH, Kirmaci HA (2010) Functional milks and dairy beverages. Int J Dairy Technol 63:1–15. doi:10.1111/j.1471-0307.2009.00547.x

Ozer D, Akin S, Ozer B (2005) Effect of inulin and lactulose on survival of Lactobacillus Acidophilus LA-5 and Bifidobacterium Bifidum BB-02 in Acidophilus-Bifidus Yoghurt. Food Sci Technol Int 11:19–24. doi:10.1177/1082013205051275

Parvez S, Malik KA, Ah Kang S, Kim H-Y (2006) Probiotics and their fermented food products are beneficial for health. J Appl Microbiol 100:1171–1185. doi:10.1111/j.1365-2672.2006.02963.x

Perrig D, Boiero ML, Masciarelli OA et al (2007) Plant-growth-promoting compounds produced by two agronomically important strains of Azospirillum brasilense, and implications for inoculant formulation. Appl Microbiol Biotechnol 75:1143–1150. doi:10.1007/s00253-007-0909-9

Picot A, Lacroix C (2004) Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. Int Dairy J 14:505–515. doi:10.1016/j.idairyj.2003.10.008

PVS Laboratories Limited Aqua Culture Products: P-Lact Plus. http://www.pvslabs.com/Shrimp.aspx#2. Accessed 8 Jan 2015

Qi Z, Zhang X-H, Boon N, Bossier P (2009) Probiotics in aquaculture of China—current state, problems and prospect. Aquaculture 290:15–21. doi:10.1016/j.aquaculture.2009.02.012

Reid G, Howard J, Gan BS (2001) Can bacterial interference prevent infection? Trends Microbiol 9:424–428

Rokka S, Rantamäki P (2010) Protecting probiotic bacteria by microencapsulation: challenges for industrial applications. Eur Food Res Technol 231:1–12. doi:10.1007/s00217-010-1246-2

Ross RP, Fitzgerald G, Collins K, Stanton C (2002) Cheese delivering biocultures—probiotic cheese—Search. Aust J Dairy Technol 57:71–78

Roy D, Mainville I, F M (1997) Bifidobacteria and their role in yogurt-related products. Microecol Ther 26:167–180

Saarela M, Alakomi H-L, Mättö J et al (2011) Acid tolerant mutants of Bifidobacterium animalis subsp. lactis with improved stability in fruit juice. LWT Food Sci Technol 44:1012–1018. doi:10.1016/j.lwt.2010.11.004

Saleem M, Arshad M, Hussain S, Bhatti AS (2007) Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J Ind Microbiol Biotechnol 34:635–648. doi:10.1007/s10295-007-0240-6

Salminen MK, Tynkkynen S, Rautelin H et al (2002) Lactobacillus bacteremia during a rapid increase in probiotic use of Lactobacillus rhamnosus GG in Finland. Clin Infect Dis 35:1155–1160. doi:10.1086/342912

Salminen S, Wright A Von, Morelli L et al (1998) Demonstration of safety of probiotics—a review. 44:93–106

Sanders ME, Klaenhammer TR (2001) Invited review: the scientific basis of Lactobacillus acidophilus NCFM functionality as a probiotic. J Dairy Sci 84:319–331. doi:10.3168/jds.S0022-0302(01)74481-5

Sazawal S, Hiremath G, Dhingra U et al (2006) Effi cacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled. 6

Seenivasan C, Radhakrishnan S, Shanthi R et al (2014) Effect of Lactobacillus sporogenes on survival, growth, biochemical constituents and energy utilization of freshwater prawn Macrobrachium rosenbergii post larvae. J Basic Appl Zool 67:19–24. doi:10.1016/j.jobaz.2013.12.002

Sekhon BS (2010) Food nanotechnology—an overview. Nanotechnol Sci Appl 3:1–15

Seo JK, Kim SW, Kim MH et al (2010) Direct-fed microbials for ruminant animals. Asian-Australasian J Anim Sci 23:1657–1667. doi:10.5713/ajas.2010.r.08

Shah NP, Lankaputhra WEV, Britz ML, Kyle WSA (1995) Survival of Lactobacillus acidophilus and Bifidobacterium bifidum in commercial yoghurt during refrigerated storage. Int Dairy J 5:515–521. doi:10.1016/0958-6946(95)00028-2

Sheng X-F, Xia J-J, Jiang C-Y et al (2008) Characterization of heavy metal-resistant endophytic bacteria from rape (Brassica napus) roots and their potential in promoting the growth and lead accumulation of rape. Environ Pollut 156:1164–1170. doi:10.1016/j.envpol.2008.04.007

Shilovskya TE (1983) No Title. Diary Sci Abstr 45:3644

Singh K, Kallali B, Kumar A, Thaker V (2011) Probiotics: a review. Asian Pac J Trop Biomed S287–S290

Snydman DR (2008) The safety of probiotics. Clin Infect Dis 46(Suppl 2):S104–11; discussion S144–51. doi:10.1086/523331

Soccol CR, Porto L, Vandenberghe DS et al (2013) The potential of probiotics: a review. 48:413–434

Song D, Ibrahim S, Hayek S (2012) Recent application of probiotics in food and agricultural science. 3–36

Sukahan feed additive SUKAFEED-B.Sub. http://weifangsukahan.en.china.cn/selling-leads/detail,1136598945,feed-additive-SUKAFEEDBSub.html. Accessed 8 Jan 2015

Surono I, Hosono A (2011) Fermentation milks: types and standards of identity. In: Fuquay JW, Fox PF, McSweeney PLH (eds) Encycl Dairy Sci, 2nd edn. Academic Press, p 4170

Szajewska H, Urbańska M, Chmielewska a et al (2014) Meta-analysis: Lactobacillus reuteri strain DSM 17938 (and the original strain ATCC 55730) for treating acute gastroenteritis in children. Benef Microbes 5:285–293. doi:10.3920/BM2013.0056

Tareb R, Bernardeau M, Gueguen M, Vernoux J-P (2013) In vitro characterization of aggregation and adhesion properties of viable and heat-killed forms of two probiotic Lactobacillus strains and interaction with foodborne zoonotic bacteria, especially Campylobacter jejuni. J Med Microbiol 62:637–649. doi:10.1099/jmm.0.049965-0

TrendMonitor (2013) Product spotlight: inner eco coconut water probiotic kefir. http://trndmonitor.com/product-spotlight-inner-eco-coconut-water-probiotic-kefir/. Accessed 8 Jan 2015

UC Davis (2014) Probiotic decreases the risk of necrotizing enterocolitis. http://www.ucdmc.ucdavis.edu/publish/news/newsroom/9336. Accessed 8 Jan 2015

Vasiljevic T, Shah NP (2008) Probiotics—from metchnikoff to bioactives. Int Dairy J 18:714–728. doi:10.1016/j.idairyj.2008.03.004

Vedamuthu ER (2006) Other Fermented and culture-containing Milks, First. Manuf Yogurt Fermented Milks 295–308. doi:10.1002/9780470277812

Vidhyalakshmi R, Bhakyaraj R, Subhasree RS (2009) Encapsulation “the future of probiotics”—a review. Adv Biol Res (Rennes) 3:96–103

Vine NG, Leukes WD, Kaiser H (2004) In vitro growth characteristics of five candidate aquaculture probiotics and two fish pathogens grown in fish intestinal mucus. FEMS Microbiol Lett 231:145–152. doi:10.1016/S0378-1097(03)00954-6

Wegmans Inner-eco Kefir, Probiotic, Dairy-Free, Original-Wegmans. http://www.wegmans.com/webapp/wcs/stores/servlet/ProductDisplay?productId=771625&storeId=10052&langId=-1. Accessed 08 Jan 2015

Wong S, Jamous A, O’Driscoll J et al (2014) A Lactobacillus casei Shirota probiotic drink reduces antibiotic-associated diarrhoea in patients with spinal cord injuries: a randomised controlled trial. Br J Nutr 111:672–678. doi:10.1017/S0007114513002973

Yakult Yakult Probiotic milk. http://yakultusa.com/#products. Accessed 13 Mar 2015

Chapter 4
Microencapsulation Technologies

Mary Ann Augustin and Luz Sanguansri

Abstract Microencapsulation involves the packaging of a gaseous, liquid or solid substance (i.e., the core or active) within a secondary material in small capsules in the range of about 0.5−2000 μm. Microencapsulation protects and stabilizes the encapsulated substance until it is released at a desired site and time by conditions that trigger its release from the microcapsule. By appropriate formulation and processing, microencapsulated ingredients may be designed to achieve the desired properties that make them superior to the neat bioactive core in the intended application. The design of a microencapsulated ingredient requires a multidisciplinary approach that includes considering the physico-chemical properties of the core and the materials to be used as encapsulants, the design and formulation of the microencapsulated ingredient, and the choice of technology for processing the microcapsules. The technologies available for the microencapsulation of various food bioactives and the properties of selected microencapsulated ingredients are discussed.

4.1 Introduction

Microencapsulation may be defined as a technology that involves the packaging of a gaseous, liquid or solid substance (i.e., the core or active) within a secondary material (i.e., matrix, encapsulant) in small capsules in the range of about 0.5−2000 μm. Microencapsulation protects and stabilizes the encapsulated substance and allows controlled delivery of the encapsulated substance at a desired site and rate when the capsule is exposed to specific conditions which provide a trigger or stimulus for the breakdown of the capsule. Many types of microencapsulated formulations can be developed for the delivery of food bioactives and desirable microorganisms. For food

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applications, the whole delivery system has to be created from food grade ingredients. The inherent properties of the bioactive and the encapsulating material, including their solubility in oil and aqueous systems and their affinity for interfaces, their resistance to degradation in various environments (e.g., when exposed to oxygen, high temperature, enzymes), and the interactions between the bioactive and the encapsulant matrix are some of the major factors that govern the choice of the formulation and the technology for the processing of the microencapsulated bioactive (Augustin and Hemar 2009; Garti and McClements 2012; Nazzaro et al. 2012).

Microencapsulation technologies have been used in the pharmaceutical and chemical industries for some time. Microencapsulation is comparatively new to the food industry, but as there are similar requirements for stabilization and controlled delivery in the food and pharmaceutical industries, the food industry has adapted many of the technologies from the pharmaceutical and chemical industries when developing microencapsulated food ingredients. However, a factor that needs to be taken into account when adapting the technology for commercial application in the food industry is the low margins in the food industry compared to the pharmaceutical and fine chemical industries, which can tolerate higher cost technologies. Another difference is that the pharmaceutical and chemical industries have the option to use a wider range of materials and chemical polymers as encapsulants, as they are not restricted to food materials or materials that have GRAS (generally regarded as safe) status. Selecting the appropriate process for the production of microcapsules requires consideration of (1) technical issues, such as the inherent properties of the core, the encapsulant matrix, the size and morphology of the particle, the mechanisms triggering the release of the core and the format of delivery; (2) the cost, consistency and sustainability of the raw materials and formulation; and (3) the economics of the microencapsulation technology, increasingly including the associated environmental footprint of the process.

The focus of this chapter is the methods used for the processing of microcapsules and the process variables that can be manipulated to achieve the desired properties of the microcapsules. However, the method of choice for processing microcapsules cannot be detached from the materials and formulation used for the design of the systems. Therefore some aspects of formulation are also considered from the perspective of material-process interactions for improving the amenability of the formulation to be transformed into a microencapsulated ingredient in the desired format with the required functional performance.

4.2 Ingredients Used in the Formulation of Food-Grade Microencapsulated Products

The formulation of the microencapsulated product is the first consideration once the target application has been defined. Table 4.1 lists some of the major bioactives of interest to the food and nutraceutical industries. Materials that have commonly been used in microencapsulated formulations as the carrier or encapsulant matrices for food bioactives include a range of proteins (e.g., caseins and whey proteins, soy

Table 4.1 Selection of bioactives of interest to the food and nutraceutical industries

Bioactive	Examples	Sources
Omega-3 fatty acids	α-linolenic acid (ALA)	Flax, perilla, chia
	Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA)	Fish oil, marine algae, krill oil
Probiotics	Lactobacilli, Bifidobacterium	Cultured microorganisms
Prebiotics	Inulin	Chicory root, Jerusalem artichoke, jicama
	β-glucan	Barley, oats
Carotenoids	β-carotene	Carrots, sweet potato, palm oil, algae
	Lycopene	Tomato, water melon, red grapefruit
	Lutein and zeaxanthin	Nasturium (yellow flowers), kale, spinach
Phenolic compounds and polyphenols	Resveratrol	Japanese knotweed, wine
	Curcumin	Tumeric
	Flavonoid (quercetin and rutin)	Onions
	Flavonoids (hesperitin)	Orange juice
	Catechins and epicatechins	Cocoa, chocolate, tea

proteins, wheat proteins), sugars and syrups, starches, gums (e.g., gum acacia, alginate, pectin, carrageenan, cellulosic material), chitosan, oils and fats, phospholipids and food-grade low molecular weight emulsifiers (e.g., Tweens).

The development of a delivery system should achieve the following attributes of performance in the intended application: (1) the stabilization of the original food bioactive after isolation from a food source; (2) the protection of the bioactive against degradation when it is in an encapsulated ingredient format and when it is incorporated into a manufactured food product; (3) the masking of tastes (if required; for example, with bioactives such as polyphenols which have an astringent tastes) so that the fortified food product has sensory appeal and is acceptable to the consumer; and (4) the delivery of the bioactive to the intended site in the gastrointestinal tract after consumption. The ability to meet all the demands of performance of a delivery system presents several scientific and technical challenges in developing a successful delivery system for a bioactive. When more than one bioactive is to be co-delivered in one encapsulation system, the challenges are even greater depending on the inherent physical and chemical properties of the bioactives, as well as the interactions between them and with the matrix.

Depending on the technology or method used for microencapsulation, different formats, morphologies, structures and other properties can be produced. The desired properties and functionality can be tailored to suit the target application. Therefore, it is important to define the criteria that will define a successful microencapsulated ingredient. The defined criteria allow the proper selection of the microencapsulation technology. Each of the available technologies can create microcapsules within a

Table 4.2 Encapsulation Processes

Physical processes	Chemical processes
Drying (Spray drying, freeze drying)	Coacervation (Simple and complex)
Spray chilling	Inclusion complexation
Fluidized bed coating	Hydrogels, beadlets
Extrusion	Biopolymeric particles
Spinning disk coating	Emulsion-based systems including layer-by-layer deposition
Supercritical fluid processing	Liposomes

particular range of size and bioactive payload type and quantity. Often, an approach based on a retro-fit design is used as a guide to the formulation and design of a microencapsulation system that is suited for the performance required in the final application (Ubbink and Krueger 2006). It can also sometimes be an iterative process where a formulation is first developed, its performance checked, and re-formulation carried out to optimize the microencapsulated formulation (Augustin and Sanguansri 2012). However, although it may appear to be deceptively simple to formulate and process a microencapsulated product with all the desirable attributes, it should be appreciated that the development of a microencapsulated product requires a multi-disciplinary approach and significant scientific knowledge of each component and process used in its development. Once the formulation has been developed, the bioactive core and the carrier matrix/encapsulant material are then made into the microencapsulated ingredient using the appropriate microencapsulation technology. The common processes employed for microencapsulation of bioactives for food application are listed in Table 4.2, and described in the following section.

4.3 Microencapsulation Technologies for Bioactive Delivery

Microencapsulation technologies generally refer to processes or methods applied to produce a microencapsulated ingredient or bioactives, with various types of morphologies and structures (Fig. 4.1). These are classified into physical or chemical processes, and often a combination of both physical and chemical processes is applied in order to achieve the desired functionality in the final microcapsule.

Depending on the technology used for microencapsulation, different formats, morphologies, structures and other properties can be produced. The desired properties and functionality can be tailored to suit the target application. The complexity of the food matrix and the stresses that the microencapsulated ingredients are exposed to during the manufacture of food make the choice of the appropriate formulation and technology more challenging. Therefore, it is important to define the criteria required for a successful microencapsulated product/ingredient (Table 4.3). These criteria allow for the proper selection of the microencapsulation technology.

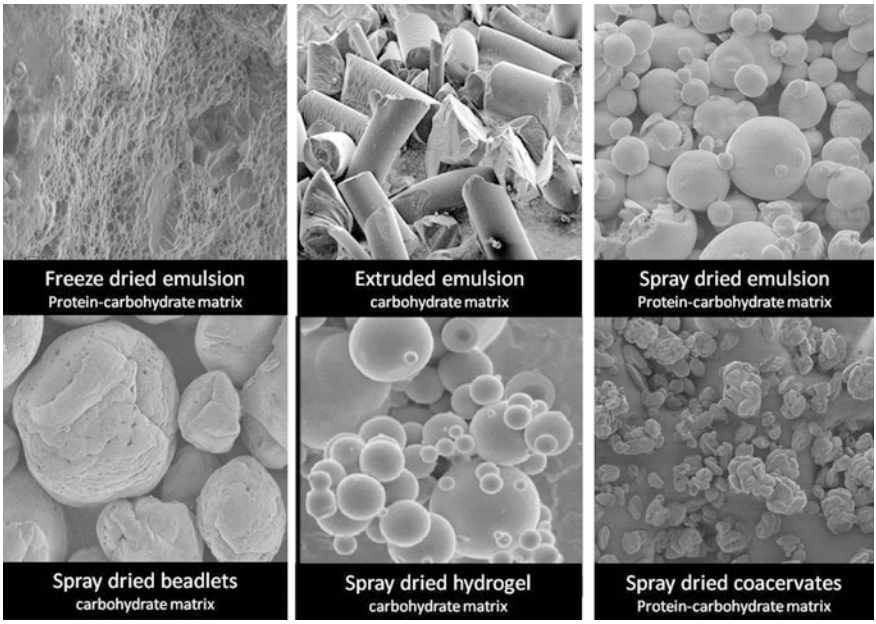

Fig. 4.1 Microstructure of dried encapsulated ingredients

Table 4.3 Criteria for selection of microencapsulation technology based on desired performance of the microencapsulated ingredient in final applications

Desired performance	Technology	Format	Particle size (μm)	Payload (%)
Liquid to powder conversion	Spray drying	Powder, granules, agglomerates	10–400	5–50
	Freeze drying	Powder	20–5000	5–50
	Spray chilling	Powder	20–200	10–20
Solubilization	Emulsion	Liquid	0.2–5000	1–20
	Nanoemulsion	Liquid	0.01–0.1	Various
Stabilization	Spray drying	Powders	10–400	5–50
	Emulsion	Liquid	0.2–5000	1–25
	Coacervation	Powder	10–800	40–90
	Inclusion complexation	Powder	0.001–0.01	5–15
	Biopolymeric particles	Liquid, powder	10–1000	20–50
	Fluid bed coating	Powder, granules, agglomerates	5–5000	5–50
Taste masking	Inclusion complexation	Powder	0.001–0.01	5–15
	Binding to biopolymers	Liquid, powder	10–1000	20–50
	Fluid bed coating	Powder, granules, agglomerates	5–5000	5–50

(continued)

Table 4.3 (continued)

Desired performance	Technology	Format	Particle size (μm)	Payload (%)
Visual clarity	Nanoemulsion	Liquid	0.01–0.1	Various
Controlled release	Liposome entrapment	Liquid, powder	10–1000	5–50
	Extruded microsphere	Beadlets, powder	200–5000	20–50
	Emulsified microspheres	Beadlets, powder	10–1000	20–50
	Nozzle coextrusion	Beadlets, powder	150–8000	70–90
	Rapid expansion of supercritical fluid	Powder	10–400	20–50
	Fluid bed coating	Powder, granules, agglomerates	5–5000	5–50
Shear resistance	Spray drying	Powder	10–400	5–50
	Extrusion	Powder, pellets	200–5000	5–40
Compression resistance	Spray drying	Powder	10–400	5–50
	Extrusion	Powder, pellets	200–5000	5−40

4.3.1 Physical Processes

4.3.1.1 Drying

Drying converts liquid materials into solid or semi-solid particles. The process results in the production of powders, granules or dry agglomerates. For food applications, powder formats are generally preferred over slurries, emulsions or suspensions. Powders are much easier to handle and transport, have longer storage stability, and may be readily added to a range of food formulations. The most common drying methods for the preparation of microencapsulated ingredients are spray drying and freeze drying.

Spray Drying

Of the drying methods, spray drying is the preferred method, as it is cost-effective and is also suitable for the drying of heat-sensitive bioactives (Gouin 2004; Murugesan and Orsat 2012).

The major stages in a spray drying process are (1) atomization of the liquid feed or slurry; (2) the contact of the atomized droplets with the drying medium; (3) the evaporation of water from the droplet; and (4) the separation of the dried product from the air. The rapid evaporation of water during spray drying keeps the product temperature relatively low (below the outlet temperature), and hence, it is possible to use spray drying technology to produce microencapsulated products with heat sensitive cores.

Spray drying has been used for microencapsulated vitamins, minerals, flavors, antioxidants (Murugesan and Orsat 2012), omega-3 oils (Augustin et al. 2006), fruit

fibers with bioactives (Chiou and Langrish 2007) and probiotics (Silva et al. 2011). The formulation (bioactive core, encapsulation material, ratio of core: encapsulant), the processes (e.g., homogenization conditions) for preparing the feed and process parameters used in spray drying, such as inlet and outlet temperatures, feed rates, feed temperature, airflow rates and type of atomizer or nozzle, all impact the properties of the final microencapsulated product (Murugesan and Orsat 2012). For example, in the preparation of spray dried lycopene with gelatin and sucrose as wall materials (core:wall ratio of 1:4 and gelatin:sucrose ratio of 3:7), it was found that increasing the feed temperature from 35 to 55 °C increased encapsulation yield and encapsulation efficiency, but further increase in the feed temperature to 65 °C was detrimental. This was possibly due to the need to balance good atomization, which improves with lower viscosity at higher feed temperatures versus the build of particles on the chamber wall due to the trajectory of the atomized particles when the feed viscosity is too low (Shu et al. 2006). These authors also showed that inlet temperature affected encapsulation yield and encapsulation efficiency within the range of inlet temperatures of 170–210 °C, and the optimum inlet temperature was 190 °C (Shu et al. 2006). However, increasing the inlet temperature from 145 to 175 °C decreased the lycopene content of spray dried watermelon powders with maltodextrin as the encapsulating agent (Quek et al. 2007).

Freeze Drying

The freeze drying process has three major stages: (1) the freezing of the liquid formulation which results in nucleation and growth of ice crystals and the formation of a glassy matrix; (2) the primary drying where the ice crystals are sublimed under low pressure; and (3) the secondary drying where the unfrozen water is desorbed by increasing the temperature or reducing the pressure during drying, depending on the material sorption isotherm. The freezing step is important as freezing affects ice formation and therefore the morphology of the product and reconstitution time. Fast freezing rates should be avoided if there is protein in the formulation. The primary drying rate affects the resistance of the dried product. The secondary drying step allows removal of water to the desired water content (Kasper and Friess 2011). As freeze drying is carried out at low temperature, the process enables high retention of volatile components and minimal degradation of heat-sensitive bioactives. Freeze drying is the most commonly used commercial method for production of dried probiotics. It has been used for the microencapsulation of many other bioactives including polyphenols, vitamins, omega-3 oils, citrus extracts, herbal extracts, spice oils and limonene.

The method of drying and the drying conditions affect the stability of the encapsulated bioactives. This was demonstrated in the early work of Desorby et al. (1997), where β-carotene encapsulated in maltodextrin was most stable to degradation when the emulsion was freeze dried compared to when it was spray dried or drum dried, with most degradation occurring in the drum-dried samples. Whether spray drying or freeze drying had a more detrimental effect on the survival of probiotic bacteria during storage was dependent on the source and strain of the

probiotic bacteria and on the encapsulation formulation (Chávez and Ledeboer 2007; Ying et al. 2010).

4.3.1.2 Spray Chilling

The spray chilling process comprises two major steps: (1) the addition of a bioactive into a molten fat, and (2) the atomization of the molten material in a heated atomizing nozzle into a refrigerated chamber, which solidifies the molten carrier material to form solid lipid microparticles. The residence time in the spray cooling chamber is short (usually a few seconds), after which the microparticles are collected. The core is typically a lipophilic bioactive, but it can also be a hydrophilic or an amphiphilic core (e.g., a peptide), and the carrier is often a lipid that is solid at room temperature (e.g., hydrogenated or fractionated edible oils with high melting points, or beeswax).

Spray chilling is a low-cost technology that is suitable for heat-sensitive ingredients such as omega-3 fatty acids, enzymes and probiotics, but it suffers from low encapsulation efficiency, and the bioactive is sometimes expelled during storage (Okuro et al. 2013). In cases where the lipid is the encapsulant, the release of the bioactive core may be triggered by increasing the temperature to above the melting point of the fat or digestion of the fat by lipases in the gastrointestinal tract after ingestion (Okuro et al. 2013). However, it is difficult to obtain delayed release greater than 30 min with a water-soluble core in a food with high water activity (Gouin 2004).

4.3.1.3 Fluidized Bed Coating

Fluid bed coating technology involves the suspension of solid particles in a moving stream of air, which may be heated or cooled. The coating is then sprayed onto the particle through an atomizer. The coating may be a molten material (e.g., fat) or a material (e.g., proteins, polysaccharides, complex formulations, emulsifiers) dissolved in a solvent (e.g., water, aqueous ethanol). Where a coating liquid is used, it is rapidly evaporated by hot air to form the outer layer of the coated particle. Where the coating is a lipid, it solidifies when in contact with the cool air as it reaches a temperature below its melting point. The particle may be passed repeatedly through the spray coating and the drying air for many coating cycles to obtain uniform coating, and in addition, multiple types of coatings may be used sequentially. This process may be used to control the coating thickness, the particle size and the release properties of the bioactive core. A pre-requisite for film coating is good adhesion properties between the coating and the core. Variables such as solvent evaporation rate, spray rate, droplet size and temperature influence the properties of the coated particle (Kuang et al. 2010). Apart from the formulation, factors such as relative humidity, fluidizing gas flow rate, coating spray rate and the composition of the fluidization gas need to be controlled for an optimized process. This also

requires an understanding of the thermodynamics of the coating process, and psychometric charts are invaluable for providing a rational basis for optimizing the coating process (Gouin 2005).

4.3.1.4 Extrusion

Extrusion—Dripping Technologies

One of the oldest methods for encapsulation is the formation of microbeads. It has been widely used for the encapsulation of cells as it results in minimal injury to cells, and in high viability of probiotic bacteria, as well as of high retention of heat sensitive bioactives (e.g., carotenes). The most common material used as the matrix for extrusion of microbeads containing bioactives is alginate. Where alginate is used, the steps involve (1) preparing an alginate solution; (2) adding the bioactive core (usually as an emulsion) into the alginate solution; and (3) dripping the resultant emulsion or suspension into a hardening bath through an orifice (e.g., needle or nozzle). Calcium chloride is generally used as the hardening solution as calcium ions cross-link the alginate molecules, forming a gel network that entraps the loaded bioactive. The microbeads can then be dried to the desired moisture. Although this process is simple, there have been difficulties in scaling up due to the slow process required for the formation of microbeads. However, advances in technologies such as laminar jet break-up technology have increased the productivity of the process and enabled a narrower dimensional range and high encapsulation efficiency. In this process, a laminar jet of a polymer solution is broken up with a vibrating nozzle (Del Gaudio et al. 2005).

Extrusion Cooking

Extrusion cooking is an energy-efficient, low-cost process that is used for producing ready-to-eat cereals and snack products. Notably, there is increasing interest in developing nutritious snacks (Brennan et al. 2013). During extrusion cooking, ingredients are mixed, cooked, texturized and formed. Oils and nutritive ingredients may be added to the formulation and these ingredients become embedded (or encapsulated) within the matrix of the extruded product.

Yilmaz et al. (2001) examined the encapsulation of sunflower oil in a starch matrix. These authors showed that the formulation and the processing conditions during extrusion influenced the properties of the microencapsulated oil. The size of the oil droplets in the extruded product decreased with increasing HLB (hydrophilic-lipophilic balance), increasing screw speed, increasing melt temperature and decreasing throughput. The release kinetics will depend on adequate mixing and dispersion of the encapsulant within the matrix (Yilmaz et al. 2001).

Sensitive nutrients such as vitamins are degraded during extrusion. The extent of degradation depends on the type of vitamin, the formulation, and the processing conditions used (Killeit 1994). Nutrient degradation during extrusion may be

reduced by introducing protected pre-encapsulated vitamin products. This has been suggested for animal feed preparations (Putnam 1986). Others have suggested that phytochemicals (β-carotene in medium-chain triglyceride oil) should be added after the plasticization of starch ingredients, to minimize exposure time to thermal and mechanical stresses, but losses are still 30 % and higher (Emin et al. 2012). However, with the introduction of modern twin-screw extruders, extrusion encapsulation is becoming an attractive alternative, as the process is flexible, and exposure to high temperatures can be avoided by introducing bioactives into the last port of the barrel (Abbas et al. 2012).

4.3.1.5 Spinning Disk Systems

Spinning disk coating uses rotational forces to create a thin film around a core. The core ingredient is suspended in a wall material and dropped onto the rotating disk which throws the droplets out towards the circumference of the disk where the wall material solidifies through drying or chilling, depending on the properties of the encapsulant material used and on the conditions. (Mason and Sparks 1987; Sparks et al. 1995). As the spinning rate can be carefully controlled, the disk process is able to yield narrow particle size distributions between 20 microns and several millimeters (Labelle 2002). For example, with spinning disk atomizers, hydrogels (alginate) and microbeads of narrow particle size (300–600 μm) distribution are obtained (Senuma et al. 2000).

4.3.1.6 Supercritical Fluid Encapsulation

With supercritical fluid encapsulation, the core material and the encapsulating material (typically a polymer) are dispersed and/or dissolved in a supercritical fluid, such as carbon dioxide. The supercritical fluid is ejected from a nozzle in the form of a spray. The carbon dioxide flashes off very rapidly, leaving residual particulate material. The solvent and anti-solvent properties of supercritical carbon dioxide can be exploited for the micronization and encapsulation of bioactive cores. Depending on the solubility of the compounds to be encapsulated, the SAS (supercritical anti-solvent) or RESS (rapid expansion of supercritical solutions) processes can be used. Santos and Meireles (2013) have recently discussed the applications of SAS for micronization of quercetin and β-carotene and RESS for the encapsulation of bixin-rich and anthocyanin-rich extracts in polyethylene glycol as the encapsulation material. Ethanol maybe used as a co-solvent. The properties of the ingredients in the formulation, the core:encapsulant ratio, the solubility of the bioactive in the supercritical fluid and the processing conditions used influence the properties of the encapsulated product. Xia et al. (2012) prepared pro-liposomes of lutein and hydrogenated phosphatidylcholine using SAS and showed that high lutein loading liposomes with encapsulation efficiency of >90 % could be produced. An interesting application is the use of supercritical carbon dioxide for the production of β-glucan aerogels for carrying flaxseed oil. The aerogels were impregnated with a

high concentration of flaxseed oil (65 %) by using supercritical carbon dioxide as the medium for mass transfer. It was suggested that an additional coating may be required to protect the oil against degradation due to the high porosity of the aerogels (Comin et al. 2012).

4.3.2 Physico-chemical Processes

4.3.2.1 Coacervation

Coacervation has been used in the food industry for encapsulation of flavors, omega-3 oils and other ingredients (Gouin 2004; Lamprecht et al. 2001). Simple coacervates are formed from one type of polymer, and those formed from two or more types of polymer (often from oppositely charged materials) are called complex coacervates. Coacervation may involve the preparation of an oil-in-water emulsion with the lipophilic bioactive being present in the oil phase. Subsequently under turbulent mixing, two liquid phases are separated under appropriate conditions. The concentrated polymer rich phase is the coacervate containing the bioactive, and the other phase is called the equilibrium solution. Coacervates are stabilized following a change in the conditions of the environment such as temperature, pH and ionic concentration. The formation of more permanent structures with well-defined sizes and shapes requires cross-linking of the biopolymer using a chemical or enzyme, and alternatively another biopolymer may be adsorbed around the droplets for stabilization (Jones and McClements 2010). The coacervates can be dried into a free flowing powder. A variation of complex coacervation involving the addition of a controlled agglomeration step and the formation of an outer shell that surrounds the agglomerates was developed by Barrow et al. (2007) for encapsulation of omega-3 oils.

Complex coacervates formed by electrostatic binding between proteins and polysaccharides (protein-polysaccharide complex) are most commonly used in the food industry (De Kruif et al. 2004). Protein-polysaccharide complexes exhibit better functional properties in a number of applications than proteins and polysaccharide combination without complexation (Schmitt et al. 1998). The use of coacervates and protein-polysaccharide complexes as delivery systems for bioactives or sensitive molecules in food is attractive due to the variety of biopolymer combinations that may be adapted to the various requirements (e.g., mechanical properties and permeability) of the delivery system (Schmitt and Turgeon 2011). For example, a gelatin-sodium alginate based polyelectrolyte complex used as a carrier for ascorbic acid has enhanced its thermal stability compared to each of the encapsulant materials on their own (Devi and Kakati 2013).

The mechanical strength and diffusion characteristics of chitosan-based coacervates can be influenced by the type of chitosan used in forming the complex. Chitosan hydroglutamate-based complexes in combination with kappa carrageenan or with alginate have higher mechanical strength than complexes based on acid soluble chitosan. In addition, higher diffusion release characteristics of a model dye

have been shown in acid soluble chitosan coacervated microcapsules, when kappa carrageenan was used compared to alginate (Pandya and Knorr 1991). Omega-3 oil loaded coacervates have been prepared with gelatin as the capsule wall followed by addition of edible salt to precipitate the gelatin rich phase (salting-out), and using transglutaminase as the cross-linking agent for hardening the capsule wall (Soeda et al. 2003). Vitamins and polyunsaturated fatty acids that are substantially insoluble in boiling water for at least 3 min have been encapsulated by cross-linking a protein, sugar and a water-soluble salt, by heating the mixture (Chaundry et al. 1992).

4.3.2.2 Inclusion Complexation

Inclusion complexes may be formed between an active and a host molecule such as cyclodextrin or starch. Inclusion complexation takes place at a molecular level and utilizes the host molecules as the encapsulating medium.

In the case of cyclodextrins, the internal surface is lipophilic, while the external surface is hydrophilic. This structure characteristic makes cyclodextrins suitable for encapsulation of less polar molecules (e.g., essential oils) into the apolar internal cavity through hydrophobic interactions (Bhandari et al. 1999). β-cyclodextrin complexes have been used for the encapsulation of oils (onion and garlic oils, omega-3 oils) to enhance their stability to temperature, light, oxidation, polymerization or double bond migration, and mask undesirable taste and odor (Dziezak 1988; Djedaini et al. 2000; Kim et al. 2000). A complex formed between DHA and cyclodextrin prepared by a twin-screw kneader has high resistance to oxidation in fishmeal paste application even without an antioxidant in the formulation (Yoshii et al. 1997). The inclusion of polyphenols in cyclodextrins improved their water solubility, especially for the less water-soluble phytochemicals (Fang and Bhandari 2010).

A new way to prepare cyclodextrin-based supramolecular systems has been reported for encapsulation of bioactive materials with altered functions in which the process of molecular assembly can be controlled (Chen and Liu 2010). The supramolecular assembly of vitamin B6 with beta-cyclodextrin was successfully prepared using different methods (kneading, co-precipitation and freeze drying), and showed that the vitamin B6 probably enters the cyclodextrin torus when forming the β-cyclodextrin-vitamin B6 inclusion complex (Borodi et al. 2009). The use of ligand-binding proteins has been explored as an alternative to cyclodextrins for inclusion complexation (Jones and McClements 2008).

Starch inclusion complexes have been used for controlled delivery of bioactives (Lesmes et al. 2008). Examples inlcude the complexation of genistein with high-amylose starch (Cohen et al. 2011) and long chain fatty acids with amylose

(Zabar et al. 2009). Hydrophobically modified starch has also been used in the molecular inclusion of polyphenols, such as curcumin (Yu and Huang 2010). The curcumin-starch complex showed a 1670-fold increase in solubility, possibly reflecting the hydrophobic interaction and hydrogen bonding between curcumin and the hydrophobically modified starch, and the encapsulated curcumin revealed enhanced in vitro anti-cancer activity compared to the free form (Yu and Huang 2010).

4.3.2.3 Liposome Entrapment

Liposomes have been used for the encapsulation and delivery of bioactive and biological agents. Liposomes stabilize bioactive materials from changes in the surrounding environment, including enzymatic and chemical modification, as well as buffering against extreme pH and changes in ionic strength. The production of liposomes using a simple low cost process without contamination of the product is of much interest to liposome producers. Different methods of liposome preparation and loading, their applications in food, cosmetics and pharmaceuticals, the main analytical methods for their characterization, and the mechanisms of liposome targeting were recently reviewed by Maherani et al. (2011). In the food industry, liposomes have been used for flavors and nutrients, as well as for antimicrobial delivery to protect food products against the growth of spoilage and pathogenic microorganisms (Taylor and Davidson 2005). While hydrophilic bioactives are typically encapsulated in their internal aqueous phase, liposomes have also been used to encapsulate lipophilic bioactives, by dissolving the bioactive in the phospholipid before water is added and sonicated to form the encapsulated material. The use of liposomes for encapsulation of antioxidants, enzymes, proteins, vitamins and minerals has been reported; however, their lack of stability under gastrointestinal conditions (low pH and enzymes), insufficient loading of bioactives and the high cost of materials remain a challenge to liposome-based encapsulation in food applications (Liu 2013).

Bioactives entrapped in liposomes are protected from oxidation and the preparation can be added directly to food products, as a dispersion, or be dried into a free flowing powder (Haynes et al. 1991). Food-approved pro-liposomes are now available, and can be used for the microencapsulation of food ingredients. They are much simpler to use, but remain expensive. Takahashi et al. (2006) successfully prepared a thermodynamically stable liposome-encapsulated curcumin (up to 85 % entrapment efficiency) from commercially available low-cost lecithin using a mechanochemical method. In an animal study, the liposome encapsulated curcumin had significantly higher absorption in the gastrointestinal tract, leading to enhanced bioavailability and bioactivity (Takahashi et al. 2009). Soy lecithin (Mohan et al. 2016) or egg lecithins (Nii et al. 2003) are food-grade materials which have been used in the formulation of liposomes. Milk-fat-globule-membrane phospholipids isolated from buttermilk have been used to prepare liposomes using ascorbic acid as a model bioactive (Farhang et al. 2012).

4.3.2.4 Biopolymeric Particles

Biopolymeric particles can be produced using food-grade proteins or polysaccharides and used as structured carriers for both hydrophilic and hydrophobic cores for controlled release and encapsulation applications (Chang and Chen 2005; Gupta and Gupta 2005; Ritzoulis et al. 2005) and for protection and delivery of probiotics (Huq et al. 2013). Biopolymeric particles or matrices may be formed by promoting self-association or aggregation of single biopolymers or by inducing phase separation in mixed biopolymer systems, for example, using aggregative (net attractive) or segregative (net repulsive) interactions (Weiss et al. 2006). Bioactives can be encapsulated in particles designed to release in response to specific environmental triggers.

Biopolymeric matrices have been produced using simple or complex coacervation methods involving proteins or protein and polysaccharide mixtures to create transparent solid matrices designed for controlled release applications (Renard et al. 2002). Biopolymer nanoparticles or microparticles can also be formed by controlled thermal treatment of electrostatic complexes of globular proteins and ionic polysaccharides. The size, charge, and stability of the biopolymer particles can be manipulated by controlling the holding temperature, holding time, initial protein concentration, polysaccharide type, protein-to-polysaccharide ratio, co-solvent composition, pH and ionic strength (Jones and McClements 2011). The use of biopolymeric particles formed by heat treatment of beta-lactoglobulin/beet pectin was investigated for application in food products as delivery systems, clouding agents, texture modifiers, or as fat droplet mimetics (Jones and McClements 2008).

Co-precipitation or freeze thaw methods have been used to produce biopolymeric particles. A co-precipitation method has been used to encapsulate quercetin using a hydrophobic protein in aqueous medium, resulting in enhanced molecular stability of the zein:quercetin particles (zein:quercetin ratio of 25:1 and 50:1 wt/wt) to alkaline pH and UV irradiation (Patel et al. 2012). The freeze–thaw method followed by alginate gelation via ionic interactions between the polymer and the cross-linking ions was used to encapsulate D-limonene in alginate/poly(vinyl alcohol) (20:80) mixture, resulting in extended release of the encapsulated D-limonene in comparison to the free aroma (Levica et al. 2011).

4.3.2.5 Emulsion-Based Systems

Emulsion-based systems are used to deliver lipophilic bioactives (oil-in-water emulsions) or hydrophilic bioactives (water-in-oil emulsions). Different emulsion systems (e.g., simple or complex emulsion structures) can be formed depending on the emulsifier and process used during emulsion preparation (Fig. 4.2). Emulsion-based systems have typically been used to encapsulate flavors, omega-3 fatty acids, carotenes, lycopene, lutein and other lipid soluble bioactives and vitamins (McClements et al. 2009). Conventional emulsions are widely used in the food industry. However, the delivery of bioactives in a stable emulsion-based

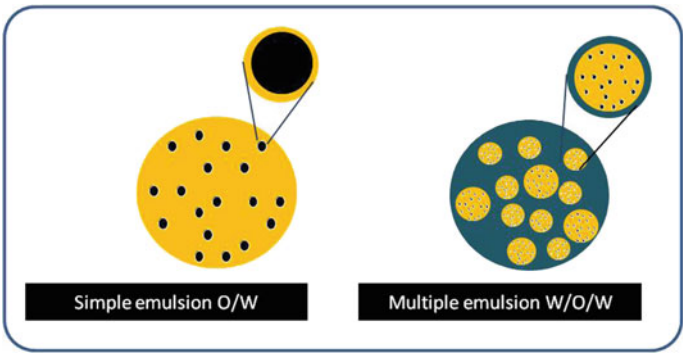

Fig. 4.2 Structure of a simple and complex emulsions

system to food and beverage may require more sophisticated design to meet the desired success criteria for particular applications. Examples of more complex emulsion based systems include multiple emulsions, micro emulsions, multilayered emulsions, solid-lipid particle emulsions, colloidosomes and internally self-assembled emulsion droplets (ISAsomes).

Simple Emulsions

A simple emulsion used for delivery is a dispersion of one immiscible phase, such as oil (containing the bioactive), in another phase (the continuous phase), such as water. During preparation of a simple oil-in-water emulsion, the oil phase is dispersed into fine droplets using an emulsifier to stabilize the oil-water interface. High shear homogenization or micro fluidization processes are used to disperse the oil into small droplets forming the emulsion. The type of emulsifier, the total solids, the pH, and the other formulation components including homogenization and storage conditions influence the emulsion properties and stability during storage (Fig. 4.3).

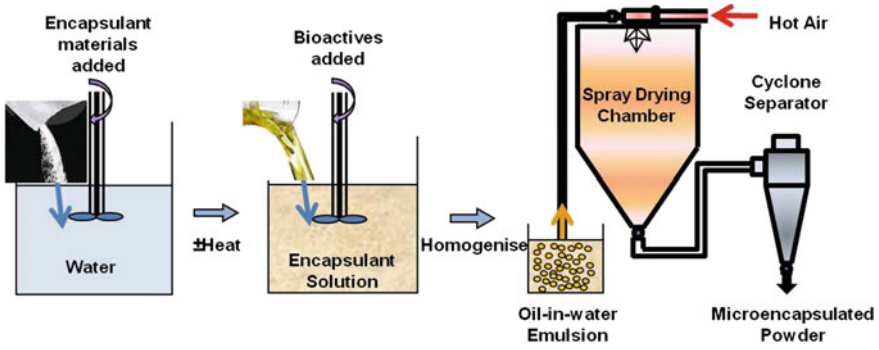

Fig. 4.3 Method for manufacture of spray dried emulsion

Multiple Emulsions

Multiple emulsions used for delivery may be in the form of water-in-oil-in-water (W/O/W) emulsions consisting of small droplets of water containing soluble bioactives, dispersed within larger oil droplets that are dispersed in an external aqueous continuous phase. W/O/W may be tailored to improve encapsulation, protection and release characteristics over simple emulsions. Bioactives can be added in both the inner water phase and the oil phase. Multiple emulsions are prepared either by one-step emulsification or by a double emulsification process. In a one-step emulsification process, the W/O emulsion is formed with the right balance of hydrophilic–lipophilic emulsifiers and heat. It can then be inverted to form a multiple emulsion. In the double-emulsification process, the primary W/O emulsions are formed under high shear conditions, and are subsequently dispersed in a secondary water phase with mild shear to avoid disruption of the already formed primary emulsions. A double emulsification and an enzymatic gelation method using transglutaminase cross-linked protein encapsulant has been suggested by Cho et al. (2003) to improve the storage stability of fish oil and achieve controlled release.

W/O/W emulsions contain a much lower amount of fat than O/W emulsions [e.g., 14 g less fat per 100 g emulsion was reported in one case (Poyato et al. 2013)], which is an aspect to be taken into account when they are used as ingredients for functional foods (Poyato et al. 2013). Although there is widespread recognition of the value of W/O/W emulsions in contributing to the development of reduced-fat products and as vehicles for the delivery of nutrients, the potential for double emulsions in food technology has yet to be fully elucidated (Dickinson 2011). de Ciriano et al. (2010) reported the need for the use of antioxidants in highly unsaturated O/W emulsions in order to control lipid oxidation. The type of antioxidant used and the phase in which it is added need careful consideration. In olive oil and linseed oil emulsions, Poyato et al. (2013) found that depending on the type of emulsions (W/O/W or O/W) the hydrophilic antioxidant (*Melissa officinalis* extract) was more efficient in O/W emulsions, whereas the lipophilic antioxidant (BHA) was more effective in W/O/W emulsions. Rutin and anthocyanins have been successfully encapsulated within the internal aqueous phase of W/O/W multiple emulsions in the (outer droplet) size range of 13−15 μm with encapsulation efficiency of >80 % (Akhtar et al. 2013).

Multilayered Emulsions

Multiple layered emulsions are emulsion produced using electrostatic layer-by-layer deposition technologies. For example, a secondary layer with an opposite electrostatic charge may be deposited onto the interfacial layer of a primary emulsion (e.g. deposition of chitosan onto the interface of droplets in lecithin-stabilized emulsions). Multilayered emulsions offer improved stability over conventional emulsions. However, their preparation is more difficult than that of conventional

emulsions as it requires extra ingredient and processing steps. Crosslinking of polymer layers adsorbed at the interface of oil-in-water emulsions is often required to enhance the stability of multilayered emulsions. Zeeb et al. (2012) demonstrated that laccase could be used to covalently crosslink pectin onto gelatin interfacial membrane, showing that pectin remained attached to the surface after increasing the pH from 3.5 to 10 and then lowering the pH again, making it robust enough for some applications. Multilayered emulsions have a thicker interfacial layer that is more resistant to disruption compared to simple emulsions. For example, lipid droplets coated by a three-component interfacial layer (β-lactoglobulin–ι-carrageenan–gelatin) were more stable to repeated freeze–thaw cycling than those coated with either a one-component (β-lactoglobulin) or two-component (β-lactoglobulin–ι-carrageenan) layer (Gu et al. 2007a). In another example, multilayered emulsions containing 1 % (w/v) κ-carrageenan with similar values for d_{32}, ζ-potential and the rheological properties at both pH values 3.5 and 7 were produced, having improved stability of against environmental stresses such as pH values around the isoelectric point of the protein (Perrechil and Cunha 2013). A protein/polyphenol microcapsule based on (-)-epigallocatechin gallate (EGCG) and gelatin (type A) was produced using the layer-by-layer (LbL) assembly containing ∼30 % w/w EGCG that retained its antioxidant activity (Shutava et al. 2009).

Multilayered emulsions formed by mixing an oil-in-water emulsion containing relatively large anionic droplets ($d_{32} \sim 0.6$ μm, β-lactoglobulin (β-Lg)–pectin coated, pH 4) with another oil-in-water emulsion containing relatively small cationic droplets ($d_{32} \sim 0.2$ μm, β-Lg coated, pH 4) were unstable to droplet aggregation at intermediate small-droplet concentrations (due to bridging flocculation) and also at high small-droplet concentrations (due to depletion flocculation); however, relatively stable particles could be made over a range of low small-droplet concentrations, which resulted in large droplets surrounded by small droplets (Gu et al. 2007b).

Solid Lipid Particle Emulsions

Solid lipid particle emulsions contain partially emulsifier-coated solid lipid particles dispersed in an aqueous continuous phase. This type of emulsion enables better control of the release of encapsulated bioactive, improved stability, higher payloads, ability to include both lipophilic and hydrophilic bioactives in the same system, and may be easier to scale up to large-scale production (McClements et al. 2009). This has originally been used in drug delivery, but has been explored more recently for delivery of bioactives in functional foods.

Internally Self-assembled Emulsion Droplets

Internally self-assembled emulsion droplets (ISAsomes) whose core comprises lipophilic molecules such as monoglycerides, are self-assembled in well-defined liquid crystalline phases. The structure of these ISAsomes can be tuned by

temperature variation and/or the addition of oils (Glatter et al. 2010). ISAsomes are potential carrier systems for hydrophilic, amphiphilic and/or lipophilic molecules. Immobilization of particles is accomplished by the addition of a polysaccharide at elevated temperatures while in a fluid state, and the system can simply be solidified by lowering the temperature at the right moment (Guillot et al. 2009). The addition of polymers (κ-carrageenan or methylcellulose) gelifies the continuous aqueous phase, forming a hydrogel loaded with ISAsomes. Self-assembled thermo-gelling emulsions were prepared by blending ISAsomes with thermo-reversible hydrogels of methylcellulose, κ-carrageenan, and their 1:1 mixture. The ISAsomes remained practically intact during their embedding into the hydrogel matrix, retaining their internal self-assembled structure and functionality (Tomsic et al. 2009). These authors found that methylcellulose was able to stabilize the ISAsomes at higher temperatures (up to 90 °C), and their most interesting results were obtained for the ISAsome-loaded (1:1) methylcellulose:κ-carrageenan system, with a narrow intermediate temperature window where it is a sol. This specific thermal behavior allows for easy temperature tuning of the system's aggregate state and of the internal self-assembled structure (Tomsic et al. 2009), offering the potential for controlled release applications.

4.4 Nanoencapsulation

Nanoencapsulation has been increasingly used for the delivery of food bioactives (Ezhilarasi et al. 2013). Nanoencapsulation typically uses micellar or vesicular systems in which the active ingredient is confined to a cavity surrounded by a unique surfactant or polymeric membrane. The preparation of nanocapsules containing a membrane forming molecule, a co-emulsifier, and a lipophilic component has been used to encapsulate a range of food ingredients, including omega-3 fatty acids. The nanocapsules were 40–80 nm in size and were suitable for delivery of components into clear liquid drinks, as well as other beverages and foods (Weder et al. 2000). Hydrophobic nanospheres of flavour compounds encapsulated in pH-sensitive or moisture-sensitive microspheres have been shown to improve shelf life of foods and beverages and prolong the sensation of flavor (Shefer and Shefer 2003).

Nanoemulsions and nanoparticles are emerging technologies that may aid in the delivery of bioactives. Nanoemulsions are submicron emulsions comprising a liquid-in-liquid dispersion of small droplets at about 20–200 nm range (Solans et al. 2005). Their small size offers potential advantages over conventional emulsions, e.g., higher stability to droplet aggregation and to gravitational separation, high optical clarity, ability to modulate product texture, and, increased bioavailability of lipophilic components (McClements and Rao 2011). Nanoemulsions may be formed using emulsifiers and very high shear resulting in emulsified fine droplets of the bioactive that is stable against sedimentation or creaming (Gonnet et al. 2010). Addition of a large amount of emulsifiers is often required; however, using high-shear homogenization helps stabilize nanoemulsions with lower levels of surfactants in the final formulation (McClements and Li 2010; Gutierrez et al.

2003). One of the difficulties in forming nanoemulsions from triglyceride oils compared to n-alkane oils is the much higher viscosity of the triglyceride oil. The size of the triglyceride oil nanoemulsion droplets was dramatically reduced by the addition of polyethylene glycol to the water phase (Wooster et al. 2008).

Nanoparticles are dense colloidal submicron particles (often polymeric), forming a homogeneous dispersion (Anton et al. 2008). Nanoparticles have been used for the delivery of a range of bioactives. The loading of hydrophobic nutraceuticals into Maillard-conjugate based nanoparticles provides protection of the nutraceuticals against degradation and enable delivery in clear beverages (Markman and Livney 2012). Lipophilic bioactives have been delivered with lipid nanoparticles to improve their bioavailability (Yao et al. 2014). Curcumin has been loaded into lipid nanoparticles (nanoemulsions), protein nanoparticles (zein nanosuspensions) and phospholipid nanoparticles (nanoliposomes) to protect curcumin from chemical degradation, increase the solubilisation of curcumin in intestinal fluids and improve oral bioavailability (Zou et al. 2016).

The future of nanoencapsulation in food requires further consideration. Consumers are concerned due to the potential and unknown possible toxicity related to nanoparticles. With the oral ingestion of nanoemulsions, there is the possibility to change the biological fate of bioactive components within the gastrointestinal tract and the potential toxicity of some of the components used in their fabrication (McClements and Rao 2011). In dry formats, nanoparticles present high risk of inhalation and may have explosive properties (Sleigh and Barton 2011). On the other hand, nanodelivery systems for nutraceuticals and other bioactives offer many advantages, including better colloidal stability, better sensory properties (suppression of undesired attributes of certain nutraceuticals), improved bioavailability, improved stability to degradation of the bioactive, and more.

4.5 Emerging Trends

Developments of microencapsulation technologies for stabilization of bioactives have been established in the last few decades. More recently, the development has focused on new microencapsulation technologies that can control the release of the bioactive at target sites in the body. Microencapsulation of bioactive components offers benefits in the development of food products with health promoting or disease preventing effects (de Vos et al. 2010). With the increasing demand for functional foods with associated health benefits, the demand for more robust microencapsulation technologies and formulations that can resist bioactive degradation during processing, shelf life and digestion are expected to grow. More robust formulations will facilitate the incorporation of bioactives into various foods and beverages.

The development of microencapsulation technologies that capitalize on applying novel combinations of different technologies to achieve the desired functionalities in the microcapsules will continue to emerge. It is, however, important to bear in mind that the more complex the technology is, the more costly it will usually be.

Spray drying and cooling have the lowest cost, fluid bed coating and spinning disk have medium cost, while coacervation and certain chemical processes have the highest cost. The question of necessity for development of more sophisticated microencapsulation technologies therefore needs to be asked. The goal of promoting human health would better be served by developing simple encapsulation technologies, and addressing bioavailability and safety concerns, to facilitate wider incorporation of nutraceuticals in food and beverages.

References

Abbas S, Wei CD, Hayat K, Xiaoming Z (2012) Ascorbic acid: microencapsulation techniques and trends—a review. Food Rev Int 28:343–374

Akhtar M, Murray BS, Afeisume EI, Khew SH (2013) Encapsulation of flavonoid in multiple emulsion using spinning disc reactor technology. Food Hydrocol 1–6. doi:10.1016/j.foodhyd.2012.12.025

Anton N, Benoit JP, Saulnier P (2008) Design and production of nanoparticles formulated from nano-emulsion templates: a review. J Controlled Release 128:185–199

Augustin MA, Hemar Y (2009) Nano- and micro-structured assemblies for encapsulation of food ingredients. Chem Soc Rev 38:902–912

Augustin MA, Sanguansri L (2012) Challenges in developing delivery systems for food additives, nutraceuticals and dietary supplements. In: Garti N, McClements DJ (eds) Encapsulation technologies and delivery systems for food ingredients and nutraceuticals. Woodhead Publishing Series in Food Science, Technology and Nutrition, Number 239, Woodhead Publishing, Oxford, pp 19–48

Augustin MA, Sanguansri L, Bode O (2006) Maillard reaction products as encapsulants for fish oil powders. J Food Sci 71(2):E25–E32

Barrow CJ, Nolan C, Jin YL (2007) Stabilization of highly unsaturated fatty acids and delivery into foods. Lipid Technol 19:108–111

Bhandari BR, D'Arcy BD, Padukka I (1999) Encapsulation of lemon oil by paste method using b-cyclodextrin: encapsulation efficiency and profile of oil volatiles. J Agric Food Chem 47:5194–5197

Borodi G, Kacso I, Fărcas SI, Bratu I (2009) Inclusion compound of vitamin B6 in b-CD. Physico-chemical and structural investigations. J Phys Conf Ser 182 012003. doi:10.1088/1742-6596/182/1/012003

Brennan MA, Derbyshire E, Tiwari BK, Brennan CS (2013) Ready-to-eat snack products: the role of extrusion technology in developing consumer acceptable and nutritious snacks. Int J Food Sci Technol 48:893–902

Chang YC, Chen DGH (2005) Adsorption kinetics and thermodynamics of acid dyes on a carboxy methylated chitosan-conjugated magnetic nano-adsorbent. Macromol Biosci 5:254–261

Chaundy FK, Bower DK, Killbride TK Jr (1992) Process for incorporating a material in a crosslinked gelatin and product there from, US Patent, US 5153177

Chávez BE, Ledeboer AM (2007) Drying of probiotics: optimization of formulation and process to enhance storage survival. Drying Technol 25:1193–1201

Chen Y, Liu Y (2010) Cyclodextrin-based bioactive supramolecular assemblies. Chem Soc Rev 39:495–505

Chiou D, Langrish TAG (2007) Development and characterization of novel nutraceuticals with spray drying technology. J Food Eng 82:84–91

Cho YH, Shim HK, Park J (2003) Encapsulation of fish oil by an enzymatic gelation process using transglutaminase cross-linked proteins. J Food Sci 68:2717

Cohen R, Schwartz B, Peri I, Shimoni E (2011) Improving bioavailability and stability of genistein by complexation with high-amylose corn starch. J Agric Food Chem 59(14):7932–7938
Comin LM, Temelli F, Saldana MDA (2012) Barley beta-glucan aerogels as a carrier for flax oil via supercritical CO_2. J Food Eng 111:626–631
de Ciriano MG, Rehecho S, Calvo MI, Cavero RY, Navarro I, Astiasarán I, Ansorena D (2010) Effect of lyophilized water extracts of *Melissa officinalis* on the stability of algae and linseed oil-in-water emulsion to be used as a functional ingredient in meat products. Meat Sci 85 (2):373–377
De Kruif CG, Weinbreck F, De Vries R (2004) Complex coacervation of proteins and anionic polysaccharides. Curr Opin Colloid Interface Sci 9:340–349
De Vos P, Faas MM, Spasojevic M, Sikkema J (2010) Encapsulation for preservation of functionality and targeted delivery of bioactive food components. Int Dairy J 20:292–302
Del Gaudio P, Colombo P, Colombo G, Russo P, Sonvico F (2005) Mechanisms of formation and disintegration of alginate beads obtained by prilling. Int J Pharm 302:1–9
Desorby SA, Netto FM, Labuza TP (1997) Comparison of spray-drying, drum-drying and freeze-drying for β-carotene encapsulation and preservation. J Food Sci 62:1158–1162
Devi N, Kakati DK (2013) Smart porous microparticles based on gelatin/sodium alginate polyelectrolyte complex. J Food Eng 117(2):193–204
Dickinson E (2011) Double emulsions stabilized by food biopolymers. Food Biophys 6(1):1–11
Djedaini PF, Perly B, Dalbiez J, Michon Q, Rousseau B, Barrier P (2000) Aqueous solution of polyunsaturated fatty acids or derivatives, solubilized and stabilised by formation of complex with gamma-cyclodextrin, used in food, cosmetic or pharmaceutical compositions, WO200053637-A1
Dziezak JD (1988) Microencapsulation and encapsulated ingredients. Food Technol 42(4):136–151
Emin MA, Mayer-Miebach E, Schuchmann HP (2012) Retention of β-carotene as a model substance for lipophilic phytochemicals during extrusion cooking. LWT-Food Sci Technol 48:302–307
Ezhilarasi PN, Karthik P, Chhanwal N, Anandharamakrishnan C (2013) Nanoencapsulation techniques for food bioactive components: a review. Food Bioprocess Technol 6:628–647
Fang Z, Bhandari B (2010) Encapsulation of polyphenols a review. Trends Food Sci Technol 21:510–523
Farhang B, Kakuda Y, Corredig M (2012) Encapsulation of ascorbic acid in liposomes prepared with milk fat globule membrane-derived phospholipid. Dairy Sci Technol 92(4):353–366
Garti N, McClements DJ (eds) (2012) Encapsulation technologies and delivery systems for food ingredients and nutraceuticals. Woodhead Publishing Series in Food Science, Technology and Nutrition, Number 239, Woodhead Publishing, Oxford 612 p
Glatter O, Kulkarni C, Chemelli A (2010) New hierarchically organized systems for delivery. Sci Pharm 78:558. doi:10.3797/scipharm.cespt.8.LDD04
Gonnet M, Lethuaut L, Boury F (2010) New trends in encapsulation of liposoluble vitamins. J Controlled Release 146:276–290
Gouin S (2004) Microencapsulation: industrial appraisal of existing technologies and trends. Trends Food Sci Technol 15:330–347
Gouin S (2005) Fluidized bed microencapsulation: thermodynamics of aqueous and ethanolic coating processes. J Microencapsul 22:829–839
Gu YS, Decker EA, McClements DJ (2007a) Application of multi-component biopolymer layers to improve the freeze–thaw stability of oil-in-water emulsions: b-Lactoglobulin–i-carrageenan–gelatin. J Food Eng 80:1246–1254
Gu YS, Decker EA, McClements DJ (2007b) Formation of colloidosomes by adsorption of small charged oil droplets onto the surface of large oppositely charged oil droplets. Food Hydrocolloids 21(4):516–526
Guillot S, Tomsic M, Sagalowicz L, Leser ME, Glatter O (2009) Internally self-assembled particles entrapped in thermoreversible hydrogels. J Colloid Interface Sci 330:175–179
Guitierrez JM, Gonzalez C, Maestro A, Sole I, Pey CM, Nolla J (2003) Nano-emulsions: new applications and optimization of their preparation. Curr Opin Colloid Interface Sci 13:245–251

Gupta AK, Gupta M (2005) Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials 26:3995–4021

Haynes LC, Levine H, Finley JW (1991) Liposome composition for the stabilization of oxidisable substances. US 5015483

Huq T, Khan A, Khan RA, Riedl B, Lacroix M (2013) Encapsulation of probiotic bacteria in biopolymeric system. Crit Rev Food Sci Nutr 53(9):909–916

Jones OG, McClements DJ (2008) Stability of biopolymer particles formed by heat treatment of β-lactoglobulin/beet pectin electrostatic complexes. Food Biophys 3:191–197

Jones OG, McClements DJ (2010) Functional biopolymeric particles: design, fabrication, and applications. Comp Rev Food Sci Food Saf 9:374–397

Jones OG, McClements DJ (2011) Recent progress in biopolymer nanoparticle and microparticle formation by heat-treating electrostatic protein–polysaccharide complexes. Adv Colloid Interface Sci 167:49–62

Kasper JC, Friess W (2011) The freezing step in lyophilization: physico-chemical fundamentals, freezing methods and consequences on process performance and quality attributes of biopharmaceuticals. Eur J Pharm Biopharm 78(2):248–263

Killeit U (1994) Vitamin retention in extrusion cooking. Food Chem 49:149–155

Kim SJ, Park GB, Kang CB, Park SD, Jung MY, Kim JO, Ha YL (2000) Improvement of oxidative stability of conjugated linoleic acid (CLA) by microencapsulation in cyclodextrins. J Agric Food Chem 48:3922

Kuang SS, Oliveira JC, Crean AM (2010) Microencapsulation as a toll for incorporating bioactive ingredients into food. Crit Rev in Food Sci Nutr 50:951–968

Labell F (2002) New, precise methods for encapsulation. Prepared Foods 55–56

Lamprecht A, Schafer U, Lehr CM (2001) Influences of process parameters on preparation of microparticle used as a carrier system for ω-3 unsaturated fatty acid ethyl esters used in supplementary nutrition. J Microencapsul 18:347

Lesmes U, Barchechath J, Shimoni E (2008) Continuous dual feed homogenization for the production of starch inclusion complexes for controlled release of nutrients. Innovative Food Sci & Emerging Technol 9(4):507–515

Levića S, Raca V, Manojlovićb V, Rakića V, Bugarskib B, Flockc T, Krzyczmonikd KE, Nedovića V (2011) Limonene encapsulation in alginate/poly (vinyl alcohol). Procedia Food Sci 1:1816–1821

Liu C (2013) Liposomes as food ingredients and nutraceutical delivery systems. Agro FOOD Ind Hi-Tech 24(2):68–71

Maherani B, Arab-Tehrany E, Mozafari MR, Gaiani C, Linder M (2011) Liposomes: a review of manufacturing techniques and targeting strategies. Curr Nanosci 7:436–452

Markman G, Livney YD (2012) Maillard-conjugate based core-shell co-assemblies for nanoencapsulation of hydrophobic nutraceuticals in clear beverages. Food Funct 3(3):262–270

Mason NS, Sparks RE (1987) Method for coating particles or liquid droplets. US4675140 A

McClements DJ, Li Y (2010) Structured emulsion-based delivery systems: controlling the digestion and release of lipophilic food components. Adv Colloid Interface Sci 159:213–228

McClements DJ, Rao J (2011) Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. Crit Rev Food Sci Nutr 51(4):285–330

McClements DJ, Decker EA, Park Y, Weiss J (2009) Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. Crit Rev Food Sci Nutr 49 (6):577–606

Mohan A, McClements DJ, Udenigwe CC (2016) Encapsulation of bioactive whey peptides in soy lecithin-derived nanoliposomes: influence of peptide molecular weight. Food Chem 213:143–148

Murugesan R, Orsat V (2012) Spray drying for the production of nutraceutical ingredients—a review. Food Bioprocess Technol 5:3–14

Nazzaro F, Orlando P, Fratianni F, Coppola R (2012) Microencapsulation in food science and biotechnology. Curr Opin Biotechnol 23:182–186

Nii T, Takamura A, Mohri K, Ishii F (2003) Factors affecting physicochemical properties of liposomes prepared with hydrogenated purified egg yolk lecithins by the microencapsulation vesicle method. Colloids Surf B-Interfaces 27(4):323–332

Okuro PK, de Matos F, Favaro-Trindada CS (2013) Technological challenges for spray chilling encapsulation of functional food ingredients. Food Technol Biotechnol 51(2):171–182 Special Issue

Pandya Y, Knorr D (1991) Diffusion characteristics and properties of chitosan coacervate capsules. Process Biochem 26:75–81

Patel AR, Heussen PCM, Hazekamp J, Drost E, Velikov KP (2012) Quercetin loaded biopolymeric colloidal particles prepared by simultaneous precipitation of quercetin with hydrophobic protein in aqueous medium. Food Chem 133:423–429

Perrechil FA, Cunha RL (2013) Stabilization of multilayered emulsions by sodium caseinate and κ-carrageenan. Food Hydrocol 30:606–613

Poyato C, Navarro-Blasco I, Calvo MI, Cavero RY, Astiasarán I, Ansorena D (2013) Oxidative stability of O/W and W/O/W emulsions: effect of lipid composition and antioxidant polarity. Food Res Int 51:132–140

Putman M (1986) Vitamin stability in livestock feed production. Feed Compounder 30–35

Quek SY, Chok NK, Swedlund P (2007) The physico-chemical properties of spray-dried watermelon powders. Chem Eng Processing 46:386–392

Renard D, Robert P, Lavenant L, Melcion D, Popineau Y, Guéguen J, Duclairoir C, Nakache E, Sanchez C, Schmitt C (2002) Biopolymeric colloidal carriers for encapsulation or controlled release applications. Int J Pharm 242:163–166

Ritzoulis C, Scoutaris N, Papademetriou K, Stavroulias S, Panayiotou C (2005) Milk protein-based emulsion gels for bone tissue engineering. Food Hydrocolloids 19:575–581

Santos DT, Meireles MAA (2013) Micronization and encapsulation of functional pigments using supercritical carbon dioxide. J Food Process Eng 36:36–49

Schmitt C, Turgeon SL (2011) Protein/polysaccharide complexes and coacervates in food systems. Adv Colloid Interface Sci 167:63–70

Schmitt C, Sanchez C, Desobry-Banon S, Hardy J (1998) Structure and technofunctional properties of protein-polysaccharide complexes: a review. Crit Rev Food Sci Nutr 38(8):689–753

Senuma Y, Lowe C, Zweifel Y, Hilborn JG, Marison I (2000) Alginate hydrogel microspheres and microcapsules prepared by spinning disk atomization. Biotechnol Bioeng 67:616–622

Shefer A, Shefer S (2003) Novel encapsulation system provides controlled release of ingredient. Food Technol 57(11):40

Shu B, Yu W, Zhao Y, Liu X (2006) Study on microencapsulation of lycopene by spray-drying. J Food Eng 76:664–669

Shutava TG, Balkundi SS, Lvov YM (2009) (_)-Epigallocatechin gallate/gelatin layer-by-layer assembled films and microcapsules. J Colloid Interface Sci 330:276–283

Silva J, Freixo R, Gibbs P, Texiera P (2011) Spray-drying for the production of dried cultures. Int J Dairy Technol 64:331–335

Sleigh S, Barton C (2011) Innovations in delivery methods for nutraceutical food and drinks. Business Insights Ltd, BI00047–001

Soeda T, Nakanishi M, Inoue T (2003) Edible microcapsules containing e.g. vitamin(s), fats and oils for addition to dried food such as soup, chewing gum and pouch-packed foods comprise edible hydrophobic core and capsule wall containing transglutaminase as crosslinking agent. US6592916-B2

Solans C, Izquierdo P, Nolla J, Azemar N, Garcia-Celma MJ (2005) Nano-emulsions. Curr Opin Colloid Interface Sci 10:102–110

Sparks RE, Jacobs IC, Mason NS (1995) Centrifugal suspension-separation for coating food ingredients. In: Risch SJ, Reineccius GA (eds) Encapsulation and controlled release of food ingredients. American Chemical Society, Washington, DC, pp 87–95

Takahashi M, Inafuku K, Miyagi T, Oku H, Wada K, Imura T, Kitamoto D (2006) Efficient preparation of liposomes encapsulating food materials using lecithins by a mechanochemical method. J Oleo Sci 56:35–42

Takahashi M, Uechi S, Takara K, Asikin Y, Wada K (2009) Evaluation of an oral carrier system in rats: bioavailability and antioxidant properties of liposome-capsulated curcumin. J Agric Food Chem 57:9141–9146

Taylor TM, Davidson PM (2005) Liposomal nanocapsules in food science and agriculture. Crit Rev Food Sci Nutr 45:587–605

Tomsic M, Guillot S, Sagalowicz L, Leser ME, Glatter O (2009) Internally self-assembled thermoreversible gelling emulsions: ISAsomes in methylcellulose, K-carrageenan, and mixed hydrogels. Langmuir 25(16):9525–9534

Ubbink J, Krueger J (2006) Physical approaches for the delivery of active ingredients in foods. Trends Food Sci Technol 17:244–254

Weder HG, Weder MA, Andreas S (2000) Use of "nanofood" in foodstuff final products for humans and animals. CA 02331661

Weiss J, Takhistov P, McClements JD (2006) Functional materials in food nanotechnology. J Food Sci 71(9):R107–R116

Wooster TJ, Golding M, Sanguansri P (2008) Impact of oil type on nanoemulsion formation and ostwald ripening stability. Langmuir 24(22):12758–12765

Xia F, Hi DD, Jin HY, Zhao YP, Liang JM (2012) Preparation of lutein proliposomes by supercritical anti-solvent technique. Food Hydrocolloids 26:456–463

Yao MF, Xaio H, McClements DJ (2014) Delivery of lipophilic bioactives: assembly, disassembly, and reassembly of lipid nanoparticles. Ann Rev Food Sci Techol 5:53–81

Yilmaz G, Jongboom ROJ, Feil H, Hennink WE (2001) Encapsulation of sunflower oil in starch matrices via extrusion: effect of the interfacial properties and processing conditions on the formation of dispersed phase morphologies. Carbohydr Polym 45:403

Ying DY, Phoon MC, Sanguansri L, Weerakkody R, Burgar I, Augustin MA (2010) Microencapsulated *Lactobacillus rhamnosus* GG powders: relationship of powder physical properties to probiotic survival during storage. J Food Sci 75(9):E588

Yoshii H, Furuta T, Kawasaki K, Hirano H, Funatsa Y, Toyomi A, Nakayama S (1997) Oxidative stability of powdery tridocosahexanoin included in cyclodextrin and its application to fish meal paste. Biosci Biotechnol Biochem 61:1376

Yu H, Huang Q (2010) Enhanced in vitro anti-cancer activity of curcumin encapsulated in hydrophobically modified starch. Food Chem 119:669–674

Zabar S, Lesmes U, Katz I, Shimoni E, Bianco-Peled, H (2009) Studying different dimensions of amylose-long chain fatt acid complexes: molecular, nano and micro level characteristics. Food Hydrocolloids 23(7):1918–1925

Zeeb B, Gibis M, Fischer L, Weiss J (2012) Crosslinking of interfacial layers in multilayered oil-in-water emulsions using laccase: characterization and pH-stability. Food Hydrocolloids 27:126–136

Zou LQ, Zheng BJ, Zhang RJ, Zhang ZP, Liu W, Liu CM, Xiao H, McClements DJ (2016) Food-grade nanoparticles for encapsulation, protection and delivery of curcumin: comparison of lipid, protein, and phospholipid nanoparticles under simulated gastrointestinal conditions. RSC Advances 6(4):3126–3136

Chapter 5
Nanoencapsulation Technologies

Yoav D. Livney

Abstract Technologies for nanoencapsulation of nutraceuticals for the enrichment of food and beverages are being developed and studied intensively, and are gradually becoming implemented by the food industry. The advances in research evidencing the health benefits of nutraceuticals, on the one hand, and the controllable risks involved in food nanotechnology on the other, have led to growing public acceptance. Consumers seek health-promoting foods that would not compromise sensory quality, or be more expensive, and comprise only natural, label-friendly ingredients. The collected knowledge and improved technological abilities generate an exciting plethora of innovative nanostructured delivery systems for nutraceuticals (e.g. nanostructured lipid carriers, nanoliposomes, co-assembled protein nanoparticles and nanofibers, and mixed polysaccharide-surfactant nanovehicles). These are formed using various novel encapsulation technologies, based on top-down, bottom-up or combined approaches. The novel nanovehicles facilitate solubilization and protection through processing, shelf life and digestion. Moreover, such nanovehicles enable programmed release and improved bioavailability of nutraceuticals, resulting in enhanced beneficial health effects.

5.1 Introduction

Over the last few decades, there is growing public awareness of the strong linkage between food and health, based on a rapidly growing body of scientific literature. As the world faces serious noncommunicable diseases, such as cardiovascular diseases, obesity, diabetes and cancer, the fast progress in food nanotechnology carries great promise to provide new and effective functional foods as tools for preventing and possibly even curing to some of these global illnesses.

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The exciting nanostructure of raw and processed food is gradually being revealed thanks to progress in nanoscopic imaging and other physico-chemical characterization techniques. This facilitates advancement in our understanding of food structure and properties, and in our ability to control and manipulate food nanostructure and to create novel and better food functionalities.

As with any revolutionary new technology, along with its great promise, food nanotechnology raises some concerns. These concerns of possible toxicity due to the much higher penetrability of nanoparticles (NPs) through biological barriers seem to have hampered progress and implementation. However, the prudent approach taken by regulators, researchers, and manufacturers, and the rising awareness of the potential benefits of these technologies, seem to be paving the way for their safe and extensive application, and for wide public acceptance.

This chapter focuses on structure and production technologies of nanodelivery systems for health-promoting bioactive compounds (nutraceuticals) in functional foods, an important and fast developing field in food nanotechnology. Most nutraceuticals cannot be incorporated in foods or beverages in their pure form. This may be attributed either to poor solubility, sensitivity to degradation during processing, shelf life and digestion, undesired sensory attributes, or poor bioavailability, and often a combination of a few of these reasons. These limitations hinder the applicability and diminish efficacy of these compounds in preventing diseases. Nanodelivery systems are designed to overcome these obstacles. Nevertheless, to be applicable and commercially viable, these delivery systems must be safe for consumption, cost effective, available on demand, simple and convenient to use, upscalable and robust, label-friendly (particularly for certain consumer groups, e.g. those with allergies, vegetarians, certain religions), and they must not adversely affect the sensory properties of the enriched product. Enhanced bioavailability, and negligible undesired sensory impact are the main advantages of nanodelivery systems.

The chapter reviews selected recently published papers in order to highlight the latest progress and to envisage potential trends for future advances.

5.2 Classification of Food Nanodelivery Systems

Numerous types of nanodelivery systems have been reported and reviewed in the literature (Cerqueira et al. 2014; Yao et al. 2014, 2015; Norton et al. 2015; Walker et al. 2015). Those studied and developed over the past 2–5 years include nanoemulsions, nanostructured lipid carriers (NLCs), Pickering emulsions, solid lipid NPs (SLN), nanosuspensions, liposomes and nanoliposomes, NPs and micelles made of proteins, polysaccharides and their complexes or conjugates, and combinations with lipid or mineral components.

Nanoemulsions (Fig. 5.1a) are colloidal dispersions of small liquid droplets, <100 nm (Gulotta et al. 2014; Walker et al. 2015). They may be oil-in-water (O/W) or water-in-oil (W/O), or even bicontinuous, and either liquid in liquid or liquid in

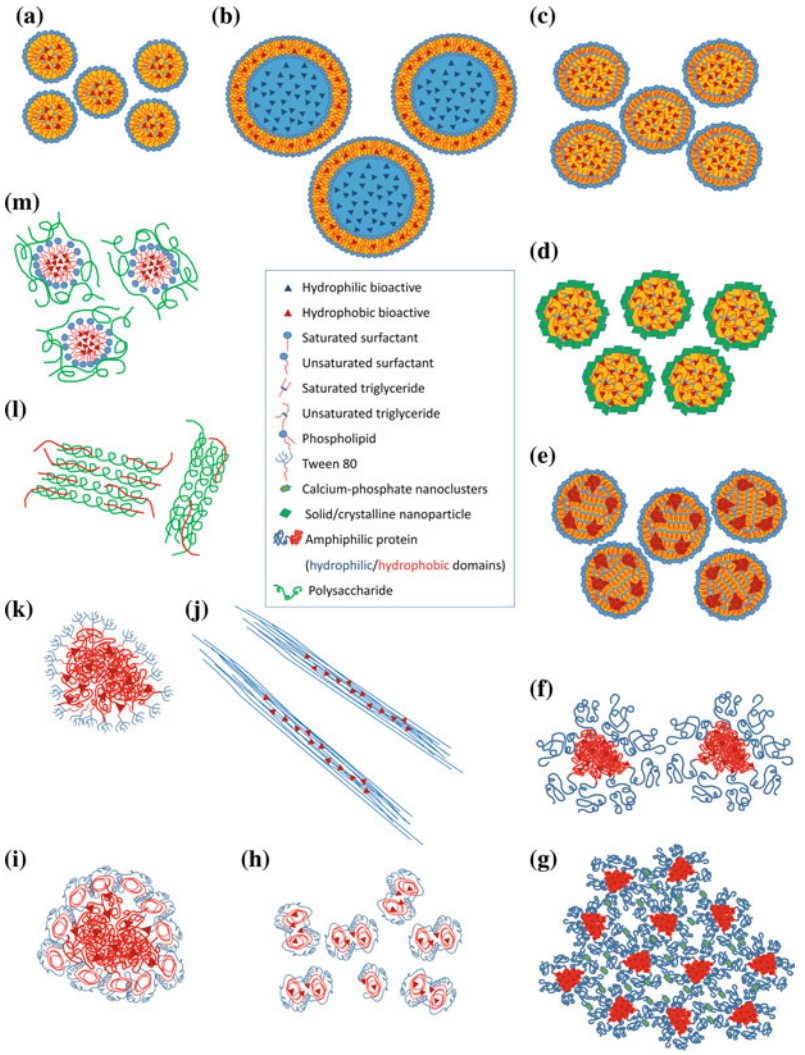

Fig. 5.1 Types of nanostructured delivery systems studied recently. **a** Nanoemulsion (liquid core, droplet size <100 nm, often <50 nm). **b** Liposomes (~200 nm to several microns) and nanoliposomes (<200 nm) (unilamellar depicted, carrying both hydrophilic and hydrophobic bioactives). **c** Nanostructured lipid carriers (NLCs) (e.g. liquid core, and crystalline shell; size <100 nm). **d** Pickering emulsion: liquid core stabilized by adsorbed solid nanoparticles. **e** Solid–lipid NPs (SLN) (a lipid nanosuspension, or a nanoemulsion with solidified droplets and bioactive; typical size <100 nm). **f** Amphiphilic biopolymer (e.g. casein) Nps (typical size <100 nm). **g** Reassembled casein micelle (size range 50–500 nm). **h** Protein-bioactive nanocomplexes (e.g. β-lactoglobulin-naringenin; size range 5–50 nm). **i** Hydrophobic protein (e.g. Zein) encapsulated by an amphiphilic one (e.g. β-lactoglobulin; size range 100–1000 nm). **j** Electrospun protein nanofibres (~500 nm thick). **k** Hydrophobic protein (e.g. Zein, ~80 nm nanoaggregate) encapsulated by a surfactant (e.g. Tween 80, ~4 nm thick nanocoat). **l** Amylose inclusion complexes entrapping fatty acids (internal structure is nanometric; particles are micrometric). **m** Polysaccharide-lipid NPs (e.g. Quercetin encapsulated in lecithin/chitosan NPs, 150–190 nm)

solid (the latter may be called solid emulsions—e.g. butter, a W/O solid emulsion). The interface may be stabilized by various types of emulsifiers, whose structure and amphiphilicity determine droplet curvature and size. Also the oil type and composition, the surfactant-to-oil ratio, and the presence of cosolvents and cosolutes may affect droplet size and emulsion stability (Komaiko and McClements 2015). The main types of amphiphiles used for nanoemulsion formation include low molecular weight surfactants, either synthetic surfactants like Tween 80 (Cho et al. 2014; Gulotta et al. 2014), Tween 20 (Salvia-Trujillo et al. 2013), and sucrose palmitate (Rao et al. 2013), or natural surfactants such as phospholipids (Lane et al. 2014) and saponins (Yang and McClements 2013); high molecular weight biopolymeric amphiphiles (Livney 2012), either synthetic like octenyl succinate modified starch (Hategekimana et al. 2014), or natural, like milk proteins (Livney 2010), particularly caseins (Penalva et al. 2015), whey proteins, mainly β-lactoglobulin (Pool et al. 2013), and milk protein peptides (Adjonu et al. 2014). Nanoemulsions are usually made either by high-shear/high-energy homogenization methods, or by low-energy, spontaneous self-emulsification methods (Piorkowski and McClements 2014; Komaiko and McClements 2015).

Liposomes (Fig. 5.1b), or vesicles, are typically spherical liquid structures with an aqueous core surrounded by a single (unilamellar) or several lipid bilayer(s) (multilamellar liposomes). Liposomes smaller than 200 nm are sometimes referred to as nanoliposomes. The main advantage of liposomes is their ability to deliver both hydrophilic and lipophilic bioactive compounds, even simultaneously. Another advantage is their similarity to natural cell membranes. However, they are quite sensitive to shear and environmental stresses, such as osmotic pressure differences, and their production scale-up is challenging. Liposomes were recently studied for their performance in the delivery of carotenoids (Tan et al. 2014), which exhibited different loading abilities into liposomes: lutein > β-carotene > lycopene > canthaxanthin. A similar trend was found for their antioxidant activity against lipid peroxidation during preparation (Tan et al. 2014). Green tea catechins, which are water-soluble bioactives, have also been encapsulated in liposomes (Rashidinejad et al. 2014). The liposomes were made of soy lecithin, and were ∼130 nm before loading, but significantly increased in size upon catechin incorporation. Loading efficiency was >70 %. The liposomes were added to the milk during low-fat hard cheese production, and almost no catechins were lost in the whey (Rashidinejad et al. 2014). Coencapsulation of lipophilic and hydrophilic nutraceuticals in unilamellar soy phospholipid-based nanoliposomes was studied using two types of commercial plant sterols—water- and oil-soluble—as well as their effect on the stability and encapsulation efficiency of ascorbic acid, a hydrophilic nutraceutical. Liposomes were prepared using high-pressure homogenization at pH 7. All liposomes obtained showed an initial monomodal size distribution with an average diameter of 115–150 nm. Incorporation of plant sterols increased the vesicle size and their encapsulation efficiency. Dilution showed content release over time (Alexander et al. 2012). Niosomes are liposomes made of nonionic surfactants. Niosomes made of a food-grade synthetic surfactant, sorbitan monostearate (Span 60), with lauryl alcohol (dodecanol) for membrane stabilization have been used for entrapping resveratrol for yogurt enrichment (Pando et al. 2015).

Nanostructured lipid carriers (NLCs) (Fig. 5.1c) are partially crystallized lipid nanovehicle particles with mean size of ≤ 100 nm, dispersed in an aqueous phase containing emulsifier(s) (Tamjidi et al. 2013). The partially solid material creates interesting nanostructures that enhance stability of entrapped bioactives, enable high loading capacity, and offer sustained-release profiles. In one study, different antioxidants (EDTA, ascorbic acid, α-tocopherol, coenzyme Q_{10}) were added to aqueous and/or lipid phases in order to observe the effect on the physical and chemical stability of NLCs (stabilized by Tween 80 and lecithin) containing astaxanthin, a hydrophobic nutraceutical. Small NPs ($\sim$94 nm) were obtained for all formulations except those with high levels of EDTA or ascorbic acid, and were stable to growth during storage at 35 °C for 15 days. EDTA and α-tocopherol increased astaxanthin oxidative stability while maintaining physical stability, with EDTA showing greater efficiency (Tamjidi et al. 2014). The influence of surfactant properties [low (LM)- and high-melting (HM) lecithins] on the physical and chemical stability of NLCs containing tristearin and omega-3 fish oil was investigated (Salminen et al. 2013), and the presence of fish oil was found to reduce the crystallization temperature, melting temperature, and melting enthalpy of tristearin. NLCs stabilized with HM-lecithin inhibited the oxidation of omega-3 fatty acids ≥ 90 % compared to those stabilized with LM-lecithin. This was attributed to the solidified surfactant layer of HM-lecithin inducing crystallization of the shell by interfacial heterogeneous nucleation (Salminen et al. 2013). Another interesting type of NLC is Pickering emulsions (Fig. 5.1d), where solid NPs are adsorbed, sometimes sintered, to the oil–water interface and provide much higher stability to emulsions than is obtainable with surfactant molecules (Dickinson 2012; Norton et al. 2015).

Nanosuspensions are systems in which solid NPs, stabilized by amphiphiles, are dispersed in a liquid medium. An interesting type of nanosuspension are solid lipid NPs (SLNs) (Fig. 5.1e), which are typically made of nanoemulsions whose dispersed phase has later been fully crystallized, and is composed of a solid carrier lipid–bioactive ingredient mixture (Salminen et al. 2013). Protein-stabilized SLNs have also been reported. One study investigated NPs containing β-carotene (BC), and encapsulated by either sodium caseinate (SC) or whey protein isolates (WPI). The BC-loaded NPs with 0.75 % SC and 1.0 % WPI were 75 and 90 nm in diameter, respectively. Sizes increased by less than 10 % at 4 °C, but by 10−76 % at 25 °C over 30 days of storage. The oxidative stability of BC was higher in SC than in WPI, and the chemical stability of BC increased as protein concentration increased (Yi et al. 2014). To enhance the apparent solubility of quercetin, three types of transglycosylated materials were studied: α-glycosyl hesperidin (Hsp-G), α-glycosyl stevia (Stevia-G) and α-glycosyl rutin (Rutin-G). The solubilizing effect of these amphiphiles decreased as follows: Rutin-G > Hsp-G > Stevia-G. The apparent solubility of quercetin from the spray-dried sample with Rutin-G was 43-fold higher than that of the quercetin crystal, likely due to the formation of molecular-level quercetin/Rutin-G nanostructured composite particles in aqueous media (Fujimori et al. 2015).

Protein/peptide nanovehicles: Curcumin was encapsulated in casein NPs (Fig. 5.1f) by spray drying their aqueous ethanol solution, which inhibited curcumin crystallinity, and obtained apparent aqueous solubility four orders of magnitude higher than with pristine curcumin (Pan et al. 2013). Curcumin was also encapsulated in native-like

phosphocasein micelles, which were improved by ultrahigh-pressure homogenization (Benzaria et al. 2013). Reassembled casein micelles (Fig. 5.1g) have been recently applied for encapsulation of β-carotene (Saiz-Abajo et al. 2013). Fish or vegetable oils have been encapsulated in casein micelles using combinations of pH modification and sonication (Ghasemi and Abbasi 2014). Whey proteins have also been studied intensively as nanovehicles for nutraceuticals. We recently formed β-Lg-naringenin nanocomplexes (Fig. 5.1h), which suppressed naringenin crystallization, for increasing its effective solubility and promoting its bioavailability (Shpigelman et al. 2014). The application of whey protein aggregation for nanocoating production was recently reviewed (Ramos et al. 2014). Lactoferrin (LF) has been studied as a vehicle for (-)-epigallocatechin-3-gallate (EGCG). Interestingly, EGCG exhibited a strong affinity for native LF, but a weak affinity for thermally modified LF at pH 5.0 and 6.5, and an inverse result at pH 3.5 (Yang et al. 2014b). Whey protein hydrolyzates were used for nanoencapsulation of caffeine, by transglutaminase cross-linking and desolvation by ethanol. Interestingly, the protective antioxidative activity of the peptides was enhanced by the transglutaminase cross-linking (Bagheri et al. 2014). Whey protein hydrolysate has also been used to chelate calcium, and the WPH-calcium chelate demonstrated excellent stability (Xixi et al. 2015). Calcium delivery by peptides was also tackled from a more unique approach, using carp egg-phosphopeptide. In vitro, egg-phosphopeptide exhibited high Ca binding ability, which was slightly lower than that of casein-phosphopeptide and could inhibit the formation of insoluble Ca salts (Huang et al. 2014). Our group recently reported employing soybean β-conglycinin (β-CG) NPs to encapsulate vitamin D (VD), thereby protecting it from thermal degradation and during shelf life (Levinson et al. 2014). Barley protein NPs (90–150 nm) have been studied as an oral delivery system for lipophilic bioactives, using high-pressure homogenization, and without organic solvents, surfactants or cross-linking agents. The NPs exhibited good colloidal stability and high loading capacity (~50 %) (Yang et al. 2014a). Omega-3-rich fish oil was encapsulated in zein via alcohol evaporation. Oil-free and fish oil-loaded zein NPs were 69 and 83 nm on average, respectively. They were added into a sugar beet pectin (SBP) solution that was subsequently gelified by the enzyme laccase (Soltani and Madadlou 2015). Tangeretin, a hydrophobic flavonoid, was encapsulated in protein NPs produced by mixing an organic phase containing zein and tangeretin with an aqueous phase containing β-lactoglobulin (Fig. 5.1i) (Chen et al. 2014).

Not only NPs, but also nanofibers made of protein have been studied as possible nanovehicles for bioactives. Electrospun zein nanofibers (~500 nm thick) loaded with fish oil were prepared (Fig. 5.1j). The lipid phase tended to concentrate at the core of the fibers, which provide a greater oxidative stability compared to non-encapsulated fish oil (Moomand and Lim 2014). Gallic acid was also incorporated into electrospun zein nanofibers (330–390 nm) as a functional ingredient. Gallic acid had retained its antioxidant activity after incorporation in the nanofibers (Neo et al. 2013).

Protein–surfactant NPs: Zein–Tween 80 NPs (Fig. 5.1k) were proposed as food-grade delivery systems. The NPs had a zein core of 78 nm diameter and a Tween 80 shell with a thickness around 4 nm (Hu and McClements 2014).

Polysaccharide-based NPs: Enzymatically synthesized dextran NPs (100–450 nm) were developed for entrapping hydrophobic nutraceuticals. Dextransucrase generated spherical dextran NPs from sucrose, which were later loaded with genistein and freeze dried (Semyonov et al. 2014). High amylose corn starch (HACS) (Fig. 5.1l) was used for encapsulating omega-3 fatty acids or silymarin for enriching bread as a functional food. Improved thermal stability of the heat labile bioactives during baking was reported (Mogol et al. 2013). Sodium alginate was used to encapsulate folic acid, using electrohydrodynamic technology to generate fine droplets (micrometers to nanometers in diameter) cross-linked in $CaCl_2$ solution (Bakhshi et al. 2013).
Polysaccharide-lipid NPs: Quercetin was encapsulated into lecithin/chitosan NPs ($\sim$170 nm) (Fig. 5.1m). Encapsulated quercetin showed improved antioxidant properties compared to free quercetin (Souza et al. 2014).

5.3 Nanoencapsulation Technologies

There are many different nanoencapsulation techniques, nanocapsule structures, and encapsulation materials, and the choice of the most suitable technology (materials, structure and technique) should be based on several considerations, including:

1. The properties of the bioactive to be encapsulated (e.g. hydrophobicity, charge), and of the intended product(s) for enrichment (e.g. water/oil-based continuous phase, liquid/solid).
2. The purpose of encapsulation, e.g. solubilization, protection (from heat, oxidizing agents, extreme pH, enzymes, etc.) during processing, shelf life, digestion; masking of undesired taste or odor; controlled release during digestion and/or facilitation of bioavailability.
3. Safety and efficacy of the technology in the intended final product(s) and any regulatory constraint.
4. Special requirements of the intended consumers [e.g. allergies, nutritional preferences like vegetarians/vegans, religious constraints (Kosher, Halal, etc.), preference for natural ingredients, etc.].
5. Intellectual property (IP) rights.
6. Availability of resources (skilled workers, equipment, materials).
7. Cost-effectiveness.

Nanoencapsulation Approaches
The process design, equipment and conditions should be chosen according to the materials used (bioactive and encapsulating materials) and the required structure, properties and physical state (e.g. liquid/powder etc.) of the desired nanocapsules. In general, the physicochemical properties such as particle size, size distribution, surface area, shape, solubility, encapsulation efficiency, and release mechanisms may be modified by the encapsulation technique (Ezhilarasi et al. 2013;

McClements 2015). Nanoencapsulation technologies may be divided into "top-down" and "bottom-up" approaches, wherein the particle size is respectively reduced or increased during the process of NP formation; however, combined approaches (McClements 2015) are also available. A top-down approach applies tools that allow size reduction and structure shaping for the desired application of the nanomaterials being developed (Ezhilarasi et al. 2013). Most top-down methods are based on mechanical forces. The major unit operations applied for mechanical size reduction are shearing/homogenization, grinding, injection/extrusion and spraying (McClements 2015). Bottom-up techniques are mostly based on physicochemical processes. The bottom-up approach is more common in nanoencapsulation, as it is generally simpler and requires less energy to form colloidally stable nano-sized particles via spontaneous self-assembly or co-assembly of molecules, than by breaking down larger structures. Bottom-up, self-assembly-based processes are, however, highly dependent on solution conditions such as temperature, pH, ionic strength etc. (Augustin and Sanguansri 2009). As an example, nanoemulsions may be produced by either a top-down or a bottom-up approach. Top-down emulsification techniques use high energy, to exert high shear on oil–water-surfactant mixtures or primary emulsions, e.g. using various homogenizers, to disrupt oil–water interface and effect droplet breakdown and size reduction down to the desired nanoscale (Abbas et al. 2013; Komaiko and McClements 2015). In contrast, bottom-up approaches for nanoemulsification require no special equipment and rely on the properties of the surfactant, oil, and water system, utilizing low energy processes, like simple mixing procedures or by changing system conditions such as temperature, to induce spontaneous formation of nanodroplets by surfactant self-assembly (Solans and Solé 2012; Schalbart and Kawaji 2013; Komaiko and McClements 2015). Combined approaches embody methods wherein some of the components are down-sized, while others are assembled, or where initial steps encompass size reduction, and later stages include nanocoating of the particles formed by co-assembly with dissolved coating materials, e.g. in layer-by-layer deposition of polyelectrolytes around emulsion droplets (Ogawa et al. 2004; McClements et al. 2009).

Another important aspect is the control of the state and internal structure of the core material. High melting point bioactives and carrier lipids tend to crystallize at storage temperature (which is an advantage for stability, but a disadvantage for bioavailability). Careful temperature control during particle formation, (e.g. heating before homogenization, and cooling afterwards) may enable the formation of solid lipid nanoparticles (SLNs) (Muller et al. 2000; Weiss et al. 2008; Aditya et al. 2014; Pandita et al. 2014; Yi et al. 2014) or partially solid, nanostructured lipid carriers (NLCs) (Tamjidi et al. 2013; Zheng et al. 2013; Aditya et al. 2014). Pickering emulsions, in which solid particles adsorb to the oil–water interface providing superb stability, may be formed by adding solid nanoparticles to either the aqueous or the oil phase (Ghouchi Eskandar et al. 2007) prior to adding the oil phase and emulsifying, but to form O/W Pickering emulsions, the solid nanoparticles should be better wetted by the aqueous phase (Dickinson 2010).

Top-Down Approaches: Reduction of Particle/Droplet Size

Size reduction may be achieved predominantly by mechanical means, which require significant energy input to overcome cohesive energy holding the molecules together in solid or liquid states, and to create larger surface area, which entails high surface energy.

(i) **High-shear/homogenization**

High shear/homogenization methods are designed and used widely for processing liquid dispersions/emulsions of various types (McClements 2015), making particle sizes smaller and more uniform. Some of the instruments used are also useful for combining and blending different streams, which is also very useful for nanoencapsulation, enabling combined top down and bottom up processes. Following are the main types of high shear/homogenization instruments useful for nanoencapsulation:

High-shear mixers are widely used to form coarse emulsions (2–10 μ) (Walstra 1993; McClements 2015), as a preliminary step before high-pressure homogenization. They are also useful for making colloidal dispersions, e.g. from phase-separated biopolymer mixtures like water-in-water (W/W) emulsions or oil-in-water-in-water (O/W/W) systems (Matalanis et al. 2010; McClements 2015). These high-shear mixers usually comprise a high-speed spinning mixing head and a container that may range in volume from a few cubic centimeters (lab scale) to several cubic meters (industrial scale) (McClements 2015). Various mixing head designs are available for different needs (Walstra 1993; McClements 2015).

High-pressure valve homogenizers (HPVHs) are perhaps the most widely used homogenizers for decreasing emulsion droplet size (McClements 2005; Lee et al. 2013), forming more uniform emulsions, and often follow a high shear mixer (McClements 2015). An HPVH usually has a piston-based pump that draws the coarse emulsion and forces it through a controllable-gap valve, where shear, cavitation, impact and turbulence break down the droplets (McClements 2005, 2015; Lee et al. 2013). Surface tension, surfactants used, and ratio of viscosities of the dispersed and continuous phases affect final droplet size distribution (Wooster et al. 2008; Lee et al. 2013). Increasing homogenization pressure, surfactant concentration and number of passes generally decrease resulting droplet size (Qian and McClements 2011). Often, a second stage of lower pressure-drop follows immediately to dissociate "flocs" formed after the first stage. HPVHs are most suitable for low to medium viscosities, and it is possible to obtain emulsion droplets <500 nm, and even <100 nm if several passes are used and a proper surfactant at sufficient concentration is present to cover the droplets (Wooster et al. 2008; McClements 2015).

Ultrasonic homogenizers harness high intensity and frequency sound waves (fluctuating pressure) to disrupt droplets and particles thus reducing their size, mainly by cavitation and microturbulence (Walstra 1993; Jafari et al. 2007; Kentish et al. 2008; McClements 2015). The two most common types are ultrasonic probe

and ultrasonic jet homogenizers (McClements 2015). The former is usually for batch, while the latter is mainly for continuous processes (Kentish et al. 2008; McClements 2015). Droplet size decreases with increasing intensity and duration of sonication, while the efficiency decreases with increasing viscosity of the phases (Jafari et al. 2007; Kentish et al. 2008; McClements 2015). Temperature rise is a problem in this technique; hence, sonication is often applied in a series of short bursts (McClements 2015).

Microfluidizers are ultrahigh-pressure homogenizers with unique flow path designs which either split the flow in two jets and then collide them head on (McClements 2005, 2015), or form a jet via an orifice, and direct it into through a reactor chamber, colliding it with a "dead-end" so that it reverses around the incoming jet, causing a counter-flow shear (Semo et al. 2007; Alvarez-Sabatel et al. 2015), thereby forming extremely fine droplets or nano-sized particles. The size obtained, which may be <100 nm, depends on the flow design, the pressure, the number of passes, and on the composition (mainly type and concentration of emulsifier used) (Wooster et al. 2008; Qian and McClements 2011). Microfluidizers are suitable for low to intermediate viscosity fluids (McClements 2015). Beyond emulsions, microfluidizers may be used for other types of colloidal systems, such as biopolymer-based systems (Panagiotou et al. 2008), and in certain instruments, it is possible to combine two streams just before homogenization, which is very useful for entrapping bioactives in nanocarrier material (Semo et al. 2007), for example, by antisolvent-induced precipitation methods, or to form a microemulsion without the need to form a coarse pre-emulsion (McClements 2015).

Microchannel homogenizers are still used mainly in research due to challenging scale-up. They are useful for creating uniform-sized and well-defined internally structured emulsions, by directing two or more streams via geometrically precise microchannels—for example, concentric channels forming core–shell microcapsular or nanocapsular structures. Droplets as small as 300 nm may be formed (Nisisako 2008; Neethirajan et al. 2011; Vladisavljević et al. 2012; McClements 2015).

Membrane homogenizers are also mainly used for research, due to the difficulties in scale-up. They are useful for creating very uniform droplets even directly from separate oil and water flows, or for reducing droplet size of a coarse pre-emulsion (Nazir et al. 2010; McClements 2015). The emulsion is formed by forcing the eventual discontinuous phase through a rigid membrane of uniform pore size into the continuous immiscible phase, which usually contains an emulsifier. The droplet size depends on the pore size, the emulsifier type and concentration, the pressure drop across the membrane, and the flow profile of the continuous phase (McClements 2015). Membrane (and also microchannel) homogenizers may also be used to form other types of colloidal particles, besides emulsions. For example, hydrogel beads may be formed by preparing a W/O pre-emulsion containing a gelling agent in the water phase, passing it through the membrane to reduce droplet size, then changing the conditions to induce gelation in the micro/nanodroplets (Zhou et al. 2008; McClements 2015).

(ii) **Spraying/atomization**
Spraying and atomization usually refer to the formation of droplets by forcing a liquid to break and disperse into fine droplets in a gas phase (Peltonen et al. 2010).

Spray drying has for many years been one of the most widely used industrial methods for drying liquids into powders and for encapsulation of active ingredients (Vega and Roos 2006; Paudel et al. 2013; McClements 2015). The principle of spray drying used for encapsulation is pumping a solution or dispersion of the active compound containing the wall material, usually in the continuous phase, through an atomizer (e.g. a nozzle, a vibrating mesh (Lee et al. 2011), an ultrasonic nozzle (Semyonov et al. 2011) or a spinning disk), which converts the liquid into a fine mist sprayed into a drying chamber, where hot air usually flows (co-current or counter-current with respect to the falling droplets), evaporating the solvent (usually water) from the droplets, and thereby converting them into small powder particles (typically 10–1000 μ). A novel Nano Spray Dryer equipped with a vibrating mesh spray technology and an electrostatic particle collector was reported to form spherical BSA NPs of about 460 nm in diameter (Lee et al. 2011). The powder is separated from the gas by gravity, filtration or centrifugally by cyclones (Vega and Roos 2006; Paudel et al. 2013; McClements 2015). Further processing may be also performed, e.g. coating or agglomeration (Fang and Bhandari 2012). There are several advantages to converting a liquid form of an active material into a powdered solid form: storage stability increases thanks to the solid state and the protective wall material, as well as improvement of handling and utilization properties, and reduced storage and shipping costs. Spray drying in particular is a rather mild drying process, which can be used for encapsulating heat-sensitive materials [particularly when done under low pressure, which may even enable survival of probiotic bacteria (Semyonov et al. 2011)], because the droplet temperature rise is suppressed by the latent heat of evaporation until solidification (Fang and Bhandari 2012; McClements 2015).

Spray chilling uses similar equipment to spray drying, but sprays a hot liquid (like molten fat) into a cold chamber to induce droplet solidification (Gamboa et al. 2011; Oxley 2012b; McClements 2015). This method is useful for encapsulation with lipids whose melting points range ~30–70 °C. The encapsulated compound is first dispersed or dissolved in the lipid carrier above its melting temperature. Chilling temperature must be sufficiently lower than the melting point to assure sufficient supercooling to initiate nucleation and crystallization (McClements 2015). Spray chilling may also be used for creating microgel or nanogel particles based on cold-setting polymers (Oxley 2012b). Spray chillers are often combined with fluidized bed coolers for a second stage cooling, and additional agglomeration or coating is also possible (McClements 2015).

Electrospraying utilizes electrical voltage to accelerate the sprayed droplets from the nozzle to a collecting electrode-plate, thereby helping to break them into much smaller droplets, which rapidly dry by evaporation before reaching the collecting plate. Particle size may be controlled by adjusting flow rate, applied voltage, and solution composition (Jaworek 2008; McClements 2015).

(iii) **Milling**
Breaking down bulk solid matrices may be a useful step in the formation of certain colloidal delivery systems. This may be achieved by several mechanical milling devices (Hu et al. 2004; Peltonen and Hirvonen 2010; Muller et al. 2011; McClements 2015). Milling may be done on dry bulk or powdered solids, or on solid in liquid dispersions. Mechanical milling devices, depending on design, exert shear, attrition, compression, or impact forces and their combinations, to reduce particle size, and they vary in the type and amount/capacity of processable materials, minimal particle size obtainable, equipment and operating costs, etc. (McClements 2015). Examples of milling devices include hammer mills, ball mills, pearl (bead) mills, cylindrical roll mills, and colloid mills (Muller et al. 2011; Bolenz and Manske 2013; Burmeister and Kwade 2013; McClements 2015).

Colloid mills are useful for dispersions of solids or liquids in viscous liquids systems (Walstra 1993; McClements 2005, 2015). They are typically made of a rotor and a stator with a gap between them that becomes narrower with the direction of flow. Usually a coarse emulsion is fed in, as they are inefficient in blending separate oil and water phases (McClements 2015). As with homogenization, a surface stabilizer or emulsifier is usually needed during milling to stabilize the newly formed surfaces and prevent aggregation (McClements 2015).

(iv) **Extrusion**
Extrusion, in the production of microparticles or NPs, encompasses the injection of a liquid through one or more thin nozzles to form small droplets, and then to fix/solidify and/or encapsulate them to form stable particles (Hu et al. 2004; Oxley 2012a; McClements 2015). For example, a solution of a polymer (possibly containing emulsion droplets of a bioactive) may be extruded into a solidifying solution, e.g. calcium ions for physical cross-linking of alginate, glutaraldehyde for chemical cross-linking of a protein, or transglutaminase for enzymatic cross-linking of a protein, or by cooling, e.g. to induce agar gelation, or to induce molten lipid crystallization (Hu et al. 2004; Oxley 2012a; McClements 2015). Co-extrusion of the core and the coating in a concentric extrusion device may be used as an elegant way to form capsular structures, and simultaneous extrusion through numerous parallel extrusion nozzles may facilitate scale-up (Oxley 2012a). Microfluidic devices may facilitate versatile formation of particles of well-defined dimensions and internal structure (Helgeson et al. 2011; Desmarais et al. 2012; McClements 2015).

(v) **Coating**
Top-down coating may be applied to confer protection, to shield and separate the core materials from the surroundings or from possible reactants, to mask undesirable flavors/odors of the core, or to control the digestive release and the

bioavailability of the coated bioactives. Some (bottom-up) coating is obtained spontaneously during particle formation—e.g. when surfactants are present during homogenization or milling. Coextrusion, mentioned above, is generally a top-down coating method. Spray coating is another top-down coating technique, in which a coating liquid (a solution, a suspension or a hot melt) is sprayed onto particles, which are usually suspended by air flow in a fluidized bed set-up, followed by hardening of the coating by drying or cooling. This method is quite limited to microencapsulation, as the core particle size is in the range of 50–500 μ (Gibbs 1999; Oxley 2012b; McClements 2015).

(vi) **Bottom-Up Approaches**
Various bottom-up approaches for nanoencapsulation have been developed over the last few decades. These techniques harness fundamental understanding of physical chemistry, to induce self-assembly and co-assembly of molecules by manipulating intermolecular forces and entropic effects by selecting molecules of desired structures and properties, and by carefully controlling the composition (bioactives, encapsulation materials and solvents), the order of mixing and the process conditions (pH, ionic strength, temperature, pressure, mixing turbulence, etc.).

- **Bottom-up emulsification methods**

Spontaneous emulsification (SE): E.g. titrating a mix of oil and a water-soluble surfactant into an aqueous solution (water + cosolvent, like ethanol) while stirring (Saberi et al. 2013; McClements 2015).
Emulsion inversion point (EIP): E.g. titrating water into a mixture of oil and a water-soluble surfactant while stirring. First, a W/O emulsion is formed, then an O/W/O, then a W/O emulsion (Ostertag et al. 2012; McClements 2015).
Phase inversion temperature (PIT): E.g. a mixture of a nonionic surfactant, oil and water is heated to just above its PIT, and then is quench-cooled while stirring, to form a nanoemulsion (Anton and Vandamme 2009; McClements 2015). Above the PIT the surfactant is more soluble in the oil phase, and forms a curvature favorable for W/O emulsion. At the PIT curvature is 0, and further cooling results in O/W as the surfactant head becomes more hydrophilic. In all the three methods above, lipophilic bioactives are added initially into the oil-phase (McClements 2015).

- **Bottom-up polymeric NP formation**
Polymer-based NPs may be formed by a variety of methods, and the appropriate polymer(s) and formation method should be chosen based on the properties of the bioactive to be encapsulated (solubility, charge, etc.), the goals of encapsulation (e.g. solubilization, protection, taste masking), the properties of the considered polymer(s) and the expected interaction mechanisms between the bioactive and the polymer (hydrophobic and van der Waals, electrostatic interactions, etc.).

Single polymer systems may be divided into single-molecule nanovehicles and multi-molecule NPs—for example, aggregates, micelles and nanocomplexes. Proteins that have both hydrophilic and hydrophobic domains may often serve as effective nanovehicles, particularly if they have solvent-accessible hydrophobic domains. Single molecule nanovehicles act by binding the individual bioactive molecule(s), thereby solubilizing, carrying and protecting them. For example, β-Lg (Swaisgood 2001), may serve as a unimolecular vehicle (also capable of forming dimers (Kontopidis et al. 2004), tetramers and octamers (Gottschalk et al. 2003) binding one to three molecules (Kontopidis et al. 2004) of a hydrophobic bioactive, such as vitamin D (Wang et al. 1997), DHA (Zimet and Livney 2009), or naringenin (Shpigelman et al. 2014) (Fig. 5.1h). Their formation may be achieved by dissolving the hydrophobic bioactive in a water-miscible cosolvent, such as ethanol, and slowly adding it at a predetermined proportion into the polymer solution while stirring it intensively (Zimet and Livney 2009; Shpigelman et al. 2014). The concentrations of the protein and the bioactive, and the ratio between them determines whether unimolecular or multimolecular NPs would form, and the loading efficiency and capacity obtained (David et al. 2014).

More common are the various multimolecular NPs, including various nanoaggregates and co-assemblies (Israeli-Lev and Livney 2014), micelles (Fig. 5.1f, g) (Semo et al. 2007; Bargarum et al. 2009; Zimet et al. 2011; Haham et al. 2012) and nanocomplexes (Fig. 5.1h) (Shpigelman et al. 2012; David et al. 2014). A unique strategy is exemplified by reassembled casein micelles (Fig. 5.1f) (Semo et al. 2007; Zimet et al. 2011; Haham et al. 2012), which are formed from one or several types of phosphoserine containing caseins: First the hydrophobic bioactive, predissolved in an organic water-miscible solvent, like ethanol, is added into the casein solution while stirring. Then, phosphate, citrate and lastly calcium solutions are added while stirring, to simulate the original mineral composition of milk, thereby inducing reformation of casein micelles, by bridging and clustering together the initially formed protein-bioactive coassemblies (or sub-micelles), to form the reassembled casein micelles, loaded with the bioactive (Semo et al. 2007; Zimet et al. 2011; Haham et al. 2012).

The formation of most multimolecular NPs is usually by phase separation, which may be achieved using several different approaches, aimed at controlling the solubility of the polymer and the bioactive by changing solvent composition, pH, ionic strength temperature etc. to induce the co-assembly of the polymer and the bioactive from their initially soluble forms. One of the most effective approaches is the antisolvent approach and its variations. In one such approach, the polymer is initially dissolved in a good solvent, or a neutral solvent (McClements 2015). This solution is then titrated while stirring into a bad solvent (typically containing the bioactive) to induce polymer aggregation and co-assembly with the bioactive. Possibly, for a hydrophobic polymer such as zein, it can be dissolved in an organic solvent, like ethanol, together with the hydrophobic bioactive. In this case, when

adding water as the antisolvent, it should contain an amphiphile such as Tween (Hu and McClements 2014) (Fig. 5.1k), or an amphiphilic protein (Chen et al. 2014) (Fig. 5.1i), to nanocoat the zein-bioactive nanoparticles formed upon the addition of antisolvent. Alternatively, a co-solvent containing the bioactive is titrated into the polymer solution while stirring. In this case, the bioactive starts aggregating upon the cosolvent dissipation in the polymer solution, and the polymer adsorbs onto the bioactive nanoparticles formed, entrapping them, and suppressing their further aggregation and crystallization (Shapira et al. 2012).

Mixed polymer systems comprise more than one polymer, and open a plethora of possibilities for creating nanodelivery systems. When two different polymers are mixed in solution, several main scenarios are possible, depending on the interactions between them (Grinberg and Tolstoguzov 1997; Tolstoguzov et al. 1997): in the case of repulsive interactions, at low concentrations they would be co-soluble, while at high concentrations, the system would phase separate due to thermodynamic incompatibility (Grinberg and Tolstoguzov 1997), forming two phases—each rich in one of the polymers, possibly forming a water in water (W/W) emulsion, which may be applied to form delivery systems, particularly if the dispersed phase (Matalanis and McClements 2012, 2013) or the continuous phase (or both) (Norton and Frith 2001) is/are later cross-linked for enhanced stability. In the case of attractive interactions (Tolstoguzov et al. 1997; Tolstoguzov 2002, 2006) (e.g. when the polymers are oppositely charged and ionic strength is low) at low polymer concentrations, or when one of the polymers is in considerable excess, soluble complexes are formed, which may serve as bioactive nanodelivery systems (Zimet and Livney 2009; Ron et al. 2010). When mutually attracted polymers exist at higher concentrations, and at pH and ratios under which they neutralize each other's charges, they form aggregative phase-separated systems, with a polymer rich phase and a polymer poor phase. These may either be coacervates (reversible, polymer and solvent rich) or precipitates (polymer-rich, solvent-poor, practically irreversible aggregates formed when pH, ionic strength and ratio cause very high attraction) (Tolstoguzov et al. 1997; Tolstoguzov 2002, 2006; McClements 2015). Attractive interactions may be used to form multi-layer emulsions, via layer-by-layer deposition of oppositely charged polymers on charged emulsion droplets (Bertrand et al. 2000; Caruso 2001; Ogawa et al. 2004; Klinkesorn et al. 2005; Johnston et al. 2006; McClements 2015), which is an elegant technology for protecting, delivering and controlling the release of oil-soluble bioactives. More complex systems, like microclusters (Dickinson 2012) of heteroaggregated emulsion droplets (Mao and McClements 2013), and colloidosomes (Gu et al. 2007), may be formed from emulsion droplets covered with oppositely charged polymers, which are then mixed together (McClements 2015).

Supercritical fluid technologies: A supercritical fluid is a material held pressurized above its critical temperature, so that it has liquid-like density, but low viscosity and high solubilization capacity (Cocero et al. 2009). Supercritical fluid technologies

are unique bottom-up methods which harness the ability of supercritical fluids, such as supercritical CO_2, to dissolve various compounds, then induce nanoparticle formation and simultaneous solvent removal by reducing the pressure (Cocero et al. 2009). Colloidal delivery systems may be formed in several ways, using supercritical fluids as either solvent or antisolvent (Cocero et al. 2009; McClements 2015). For example, the bioactive and the coating material may be both dissolved in the supercritical fluid, and when the solution is sprayed into a low pressure chamber, both bioactive and capsule material come out of solution and spontaneously co-aggregate into solid nanoparticles in a powder form (Cocero et al. 2009; McClements 2015). Alternatively, the active ingredient and the carrier material are first dissolved in a conventional solvent, and the solution is then brought into contact with a supercritical fluid, causing co-precipitation of the core and carrier materials. This approach may also be used to form core-shell particles by depositing the shell material onto existing core nanoparticles or nanodroplets (Cocero et al. 2009; McClements 2015).

5.4 Sensory Aspects of Nanostructured Delivery Systems in Food

Perhaps the main benefit of nanodelivery systems in food is the fact that they usually evade our sensory perception, enabling enrichment of food and beverages with bioactives without compromising the sensory attributes of the original product. Due to their nanometric size, they are too small for the naked eye, but if kept below about 80 nm (and not at very high concentrations, or particle refractive index not very different from that of the solution), they barely scatter any visible light, thus maintaining transparency. Such stealth nanovehicles are valuable, e.g. for enriching clear beverages with water-insoluble bioactives, or ones of undesirable flavors or odors. SLNs, and protein complexes were reported to mask bitterness of hesperetin (Fathi et al. 2013) and EGCG (Shpigelman et al. 2012), respectively. Nanovehicles useful for transparent beverage enrichment include, for example, nanoemulsions (Gulotta et al. 2014; Piorkowski and McClements 2014), protein NPs (Levinson et al. 2014; Shpigelman et al. 2014) and protein-polysaccharide conjugates (Markman and Livney 2012). Also, mouth-feel characteristics of NPs are nonexistent, as our tongue cannot sense particulates below $\sim$10 microns. Niosomes containing resveratrol did not cause any changes in the texture of regular yogurt (Pando et al. 2015). Nanocomplexes of high amylose corn starch encapsulating omega-3 fatty acids and silymarin were incorporated into bread formulation, and up to 2.5 % had no undesirable impact on bread quality parameters and sensory attributes (Mogol et al. 2013).

5.5 Bioavailability of Bioactives in NPs

The facilitation of micronutrient bioavailability is another major advantage of NPs. This is mainly due to their nanometric size (Cerqueira et al. 2014; Oehlke et al. 2014; Ting et al. 2014; Yao et al. 2014, 2015; Norton et al. 2015). The improved oral bioavailability is obtained by several mechanisms: increasing bioactive stability during digestion; enhancing nutraceutical solubility in intestinal fluids; enhancing nutraceutical transport and absorption through or between the intestinal epithelial cells; and decreasing first-pass metabolism in the gut and liver (Oehlke et al. 2014; Ting et al. 2014; Yao et al. 2015). The materials and their physical state, the nanostructure of the vehicles and surrounding food matrix affect bioavailability. An in vitro bioaccessibility study showed that casein NPs acted as gastroresistant devices and released folic acid only under simulated intestinal conditions (Penalva et al. 2015). β-Carotene bioaccessibility in starch hydrogels containing no fat was very low ($\sim$1 %) due to its crystallinity and lack of mixed micelles to solubilize it (Mun et al. 2015). The bioaccessibility of β-carotene increased with increasing digestible oil content (Rao et al. 2013). The bioaccessibility of quercetin in nanoemulsions was higher than in either bulk oil or pure water (Pool et al. 2013). Transglutaminase cross-linking slowed down the release rate of entrapped caffeine from sub-micron particles in a simulated gastric fluid (Bagheri et al. 2014).

The absorption of calcium from whey protein hydrolysate-calcium chelate in Caco-2 cells was significantly higher than those of calcium gluconate and $CaCl_2$ (Xixi et al. 2015). β-carotene cellular uptake was significantly improved through NP delivery systems by 2.6-, 3.4-, and 1.7-fold increase, respectively, for sodium-caseinate, whey protein, and soy-protein NPs, compared to free β-carotene (Yi et al. 2014).

The oral bioavailability of folic acid when administered to rats as casein NPs was around 52 %, which was 50 % higher than from pure solution (Penalva et al. 2015). Calcium bioavailability was significantly increased in calcium-deficient rats by egg-phosphopeptide supplementation (Huang et al. 2014). Another rat study showed that the bioavailability of heptadecanoic acid and of coenzyme Q_{10} was highest when encapsulated within digestible oil droplets of smallest size (Cho et al. 2014).

In a clinical human study, we have found that bioavailability of vitamin D_3 encapsulated in reformed-casein micelles in 1 % fat milk was at least as high as in aqueous Tween 80-emulsified vitamin D_3 supplement (Haham et al. 2012). Another clinical trial demonstrated significantly higher absorption of omega-3 fatty acids from a nanoemulsion than from bulk oil (Lane et al. 2014).

5.6 Health Aspects of NP Enrichment of Food: Safety and Efficacy

The augmented bioavailability of bioactives in NPs is also an important safety concern, particularly when the added nutraceutical or the nanovehicle material may become toxic above a certain dose. This requires a prudent safety evaluation, and regulatory approval, particularly when introducing new materials that have no record of safe human consumption. Increased bioavailability in nanovehicles may require re-evaluation of Recommended Daily Allowance (RDA) and Tolerable Upper Intake Level (UL) of bioactives.

Positive results regarding safety of food protein NPs in cell culture studies have been reported (Yu and Huang 2013; Yang et al. 2014a; Yi et al. 2014). Compared with micron-sized emulsions with the same compositions, nanoemulsions did not reveal more toxicity to Caco-2 cell monolayers (Yu and Huang 2013). Phosphocasein micelles did not induce damage or major inflammation to human intestinal epithelial TC7 cells as assessed by LDH release or IL-8 secretion (Benzaria et al. 2013).

Health-promoting attributes of nanoencapsulated bioactives are being studied extensively; however, more in vivo and clinical studies of their safety and health promotion are still needed. Casein–encapsulated curcumin showed higher antioxidant activity and higher anticancer activity, as evaluated by in vitro cell proliferation assay using human colon cancer cells, than unencapsulated curcumin (Pan et al. 2013). EGCG encapsulated in casein micelles caused a decrease in proliferation of cancer cells, while in healthy cells, neither free nor encapsulated EGCG affected cell proliferation at low concentrations, and its bioefficacy was not diminished regardless of the extent of digestion (Haratifar et al. 2014). EGCG-loaded chitosan-NPs orally administered to mice implanted with subcutaneous tumor xenografts resulted in significant tumor growth inhibition, compared with free EGCG and control groups (Khan et al. 2014). Calcium content, bone mineral density and biomechanical properties were significantly higher in rats following egg-phosphopeptide supplementation compared to control (Huang et al. 2014). Conjugated linolenic acid-rich oil nanocapsules significantly reduced the blood lipids, tissue lipids and plasma viscosity in high-fat diet induced hypercholesterolemia in rats (Sengupta et al. 2015).

Table 5.1 highlights nanoencapsulated bioactives and their nanovehicles, which were reported recently.

5.7 Potential Future Directions

Novel nanoencapsulation technologies are being introduced and developed at a fast pace, and their great potential in food is being clearly established. In light of global health problems, their utilization for effective preventive nutrition and health

Table 5.1 Nanoencapsulated bioactives reported recently

Bioactive		Nanovehicle (References)
Vitamins	D	Soybean β-conglycinin NPs (Levinson et al. 2014)
	A	Nanosuspension (Campardelli and Reverchon 2015)
	B9 (Folic acid)	Nanodroplets in calcium alginate (Bakhshi et al. 2013)
Minerals	Calcium	Whey protein hydrolysate nanocomposite (Xixi et al. 2015); carp egg phosphopeptide (Huang et al. 2014)
Carotenoids	β-Carotene	Fat droplets in starch-based filled hydrogels (Mun et al. 2015); protein-stabilized SLNs (Yi et al. 2014); nanoemulsions (Rao et al. 2013; Salvia-Trujillo et al. 2013); re-assembled casein micelles (Saiz-Abajo et al. 2013)
	Astaxanthin	NLCs (Tamjidi et al. 2014)
	Lutein, β-carotene, lycopene, canthaxanthin	Liposomes (Tan et al. 2014)
(Poly) phenols	Curcumin	Casein NPs (Pan et al. 2013); phosphocasein micelles (Benzaria et al. 2013)
	EGCG	β-Lactoglobulin complexes (Shpigelman et al. 2012); Lactoferrin-based NPs (Yang et al. 2014b); liposomes (Rashidinejad et al. 2014); chitosan-based NPs (Khan et al. 2014); casein micelles (Haratifar et al. 2014)
	Quercetin	Lecithin/chitosan NPs (Souza et al. 2014); nanoemulsion (Pool et al. 2013)
	Naringenin	β-Lactoglobulin complexes (Shpigelman et al. 2014)
	Tangeretin	Zein/β-lactoglobulin NPs (Chen et al. 2014)
	Genistein	Enzymatically synthesized dextran NPs (Semyonov et al. 2014)
	Resveratrol	Nanoemulsions (Davidov-Pardo and McClements 2014)
	Gallic acid	Electrospun zein nanofibres (Neo et al. 2013)
	Silymarin	Amylose inclusion complexes (Mogol et al. 2013)
	Hesperetin	SLNs (Fathi et al. 2013)
	Eugenol	Microemulsions (nanoemulsions) (Al-Okbi et al. 2014)
Fatty acids	Omega-3	Nanoemulsions (Salminen et al. 2013; Cho et al. 2014; Lane et al. 2014; Walker et al. 2015); SLNs (Salminen et al. 2013); NLCs (Salminen et al. 2013); zein NPs (Soltani and Madadlou 2015); casein micelles (Ghasemi and Abbasi 2014); electrospun zein fibers (Moomand and Lim 2014); amylose inclusion complexes (Mogol et al. 2013)

(continued)

Table 5.1 (continued)

Bioactive		Nanovehicle (References)
	Conjugated linolenic acid	Oil nanocapsules (microemulsions) (Sengupta et al. 2015)
Peptides	Whey protein bioactive peptides	Nanoemulsions (Adjonu et al. 2014)
Other nutraceuticals	Clove oil	Microemulsions (nanoemulsions) (Al-Okbi et al. 2014)
	Coenzyme Q_{10}	Nanoemulsions (Cho et al. 2014)

promotion is imperative and inevitable. Safety concerns must be further prudently addressed by accelerated in vivo and clinical trials to provide scientific support to both regulators and producers toward broader safe application. These studies can also provide consumers with scientific evidence-based information, contributing to the growing public acceptance of food nanotechnology (Schnettler et al. 2014), and allowing this boundless potential to be realized in numerous ways in the future. These may include personalized nutrition, delivery systems based on natural-renewable sources, novel functionalities for advancing human physical and mental competence, boosting-up mood, and raising the satisfaction from healthy food. Novel technologies like 3D printing may enable the creation of innovative functional food products, incorporating nanodelivery systems and personalizing product composition, shape and sensory attributes according to our genome and personal preferences.

References

Abbas S, Hayat K, Karangwa E, Bashari M, Zhang X (2013) An overview of ultrasound-assisted food-grade nanoemulsions. Food Eng Rev 5(3):139–157

Aditya NP, Macedo AS, Doktorovov S, Souto EB, Kim S, Chang P-S, Ko S (2014) Development and evaluation of lipid nanocarriers for quercetin delivery: a comparative study of solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and lipid nanoemulsions (LNE). Lwt-Food Sci Technol 59(1):115–121

Adjonu R, Doran G, Torley P, Agboola S (2014) Whey protein peptides as components of nanoemulsions: a review of emulsifying and biological functionalities. J Food Eng 122:15–27

Alexander M, Lopez AA, Fang Y, Corredig M (2012) Incorporation of phytosterols in soy phospholipids nanoliposomes: encapsulation efficiency and stability. Lwt-Food Sci Technol 47 (2):427–436

Al-Okbi SY, Mohamed DA, Hamed TE, Edris AE (2014) Protective effect of clove oil and eugenol microemulsions on fatty liver and dyslipidemia as components of metabolic syndrome. J Med Food 17(7):764–771

Alvarez-Sabatel S, de Marañón IM, Arboleya J-C (2015) Impact of high pressure homogenisation (HPH) on inulin gelling properties, stability and development during storage. Food Hydrocolloids 44:333–344

Anton N, Vandamme TF (2009) The universality of low-energy nano-emulsification. Int J Pharm 377(1–2):142–147

Augustin MA, Sanguansri P (2009) Nanostructured materials in the food industry. Adv Food Nutr Res 58:183–213

Bagheri L, Madadlou A, Yarmand M, Mousavi ME (2014) Potentially bioactive and caffeine-loaded peptidic sub-micron and nanoscalar particles. J Funct Foods 6:462–469

Bakhshi PK, Nangrejo MR, Stride E, Edirisinghe M (2013) Application of electrohydrodynamic technology for folic acid encapsulation. Food Bioprocess Technol 6(7):1837–1846

Bargarum J, Danino D, Livney YD (2009) Nanoencapsulation of vitamin D in beta casein micelles. In: IFT 2009. Anaheim, CA, USA

Benzaria A, Maresca M, Taieb N, Dumay E (2013) Interaction of curcumin with phosphocasein micelles processed or not by dynamic high-pressure. Food Chem 138(4):2327–2337

Bertrand P, Jonas A, Laschewsky A, Legras R (2000) Ultrathin polymer coatings by complexation of polyelectrolytes at interfaces: suitable materials, structure and properties. Macromol Rapid Commun 21(7):319–348

Bolenz S, Manske A (2013) Impact of fat content during grinding on particle size distribution and flow properties of milk chocolate. Eur Food Res Technol 236(5):863–872

Burmeister CF, Kwade A (2013) Process engineering with planetary ball mills. Chem Soc Rev 42 (18):7660–7667

Campardelli R, Reverchon E (2015) alpha-tocopherol nanosuspensions produced using a supercritical assisted process. J Food Eng 149:131–136

Caruso F (2001) Nanoengineering of particle surfaces. Adv Mater 13(1):11–22

Cerqueira MA, Pinheiro AC, Silva HD, Ramos PE, Azevedo MA, Flores-Lopez ML, Rivera MC, Bourbon AI, Ramos OL, Vicente AA (2014) Design of bio-nanosystems for oral delivery of functional compounds. Food Eng Rev 6(1–2):1–19

Chen J, Zheng J, McClements DJ, Xiao H (2014) Tangeretin-loaded protein nanoparticles fabricated from zein/beta-lactoglobulin: preparation, characterization, and functional performance. Food Chem 158:466–472

Cho HT, Salvia-Trujillo L, Kim J, Park Y, Xiao H, McClements DJ (2014) Droplet size and composition of nutraceutical nanoemulsions influences bioavailability of long chain fatty acids and Coenzyme Q10. Food Chem 156:117–122

Cocero MJ, Martín Á, Mattea F, Varona S (2009) Encapsulation and co-precipitation processes with supercritical fluids: fundamentals and applications. J Supercrit Fluids 47(3):546–555

David S, Zagury Y, Livney YD (2014) Soy β-conglycinin–curcumin nanocomplexes for enrichment of clear beverages. Food Biophys 10(2):195–206

Davidov-Pardo G, McClements DJ (2014) Resveratrol encapsulation: designing delivery systems to overcome solubility, stability and bioavailability issues. Trends Food Sci Technol 38(2):88–103

Desmarais SM, Haagsman HP, Barron AE (2012) Microfabricated devices for biomolecule encapsulation. Electrophoresis 33(17):2639–2649

Dickinson E (2010) Food emulsions and foams: stabilization by particles. Curr Opin Colloid Interface Sci 15(1–2):40–49

Dickinson E (2012) Use of nanoparticles and microparticles in the formation and stabilization of food emulsions. Trends Food Sci Technol 24(1):4–12

Ezhilarasi PN, Karthik P, Chhanwal N, Anandharamakrishnan C (2013) Nanoencapsulation techniques for food bioactive components: a review. Food Bioprocess Technol 6(3):628–647

Fang Z, Bhandari B (2012) Spray drying, freeze drying, and related processes for food ingredient and nutraceutical encapsulation, Chapter 4. In: Garti N, McClements DJ (eds) Encapsulation technologies and delivery systems for food ingredients and nutraceuticals. Woodhead Publishing Limited, Oxford, Cambridge, Philadelphia, New Delhi, pp 73–108

Fathi M, Varshosaz J, Mohebbi M, Shahidi F (2013) Hesperetin-loaded solid lipid nanoparticles and nanostructure lipid carriers for food fortification: preparation, characterization, and modeling. Food Bioprocess Technol 6(6):1464–1475

Fujimori M, Kadota K, Shimono K, Shirakawa Y, Sato H, Tozuka Y (2015) Enhanced solubility of quercetin by forming composite particles with transglycosylated materials. J Food Eng 149:248–254

Gamboa OD, Gonçalves LG, Grosso CF (2011) Microencapsulation of tocopherols in lipid matrix by spray chilling method. Procedia Food Sci 1:1732–1739
Ghasemi S, Abbasi S (2014) Formation of natural casein micelle nanocapsule by means of pH changes and ultrasound. Food Hydrocolloids 42:42–47
Ghouchi Eskandar N, Simovic S, Prestidge CA (2007) Synergistic effect of silica nanoparticles and charged surfactants in the formation and stability of submicron oil-in-water emulsions. Phys Chem Chem Phys 9(48):6426–6434
Gibbs BF, Alli I, Kermasha S, Catherine N, Mulligan B (1999) Encapsulation in the food industry: a review. Int J Food Sci Nutr 50(3):213–224
Gottschalk M, Nilsson H, Roos H, Halle B (2003) Protein self-association in solution: the bovine β-lactoglobulin dimer and octamer. Protein Sci 12(11):2404–2411
Grinberg VY, Tolstoguzov VB (1997) Thermodynamic incompatibility of proteins and polysaccharides in solutions. Food Hydrocolloids 11(2):145–158
Gu YS, Decker EA, McClements DJ (2007) Formation of colloidosomes by adsorption of small charged oil droplets onto the surface of large oppositely charged oil droplets. Food Hydrocolloids 21(4):516–526
Gulotta A, Saberi AH, Nicoli MC, McClements DJ (2014) Nanoemulsion-based delivery systems for polyunsaturated (omega-3) oils: formation using a spontaneous emulsification method. J Agric Food Chem 62(7):1720–1725
Haham M, Ish-Shalom S, Nodelman M, Duek I, Segal E, Kustanovich M, Livney YD (2012) Stability and bioavailability of vitamin D nanoencapsulated in casein micelles. Food Funct 3 (7):737–744
Haratifar S, Meckling KA, Corredig M (2014) Bioefficacy of tea catechins encapsulated in casein micelles tested on a normal mouse cell line (4D/WT) and its cancerous counterpart (D/v-src) before and after in vitro digestion. Food Funct 5(6):1160–1166
Hategekimana J, Bwengye MK, Masamba KG, Yokoyama W, Zhong F (2014) Formation and stability of vitamin e enriched nanoemulsions stabilized by octenyl succinic anhydride modified starch. Int J Food Eng 10(4):633–643
Helgeson ME, Chapin SC, Doyle PS (2011) Hydrogel microparticles from lithographic processes: novel materials for fundamental and applied colloid science. Curr Opin Colloid Interface Sci 16(2):106–117
Hu K, McClements DJ (2014) Fabrication of surfactant-stabilized zein nanoparticles: a pH modulated antisolvent precipitation method. Food Res Int 64:329–335
Hu J, Johnston KP, Williams RO (2004) Nanoparticle engineering processes for enhancing the dissolution rates of poorly water soluble drugs. Drug Dev Ind Pharm 30(3):233–245
Huang H, Li B, Liu Z, Mu X, Nie R, Zeng M (2014) Effectiveness of carp egg phosphopeptide on inhibiting the formation of insoluble ca salts in vitro and enhancing ca bioavailability in vivo. Food Sci Technol Res 20(2):385–392
Israeli-Lev G, Livney YD (2014) Self-assembly of hydrophobin and its co-assembly with hydrophobic nutraceuticals in aqueous solutions: towards application as delivery systems. Food Hydrocolloids 35:28–35
Jafari SM, He Y, Bhandari B (2007) Production of sub-micron emulsions by ultrasound and microfluidization techniques. J Food Eng 82(4):478–488
Jaworek A (2008) Electrostatic micro- and nanoencapsulation and electroemulsification: a brief review. J Microencapsul 25(7):443–468
Johnston APR, Cortez C, Angelatos AS, Caruso F (2006) Layer-by-layer engineered capsules and their applications. Curr Opin Colloid Interface Sci 11(4):203–209
Kentish S, Wooster TJ, Ashokkumar M, Balachandran S, Mawson R, Simons L (2008) The use of ultrasonics for nanoemulsion preparation. Innovative Food Sci Emerg Technol 9(2):170–175
Khan N, Bharali DJ, Adhami VM, Siddiqui IA, Cui H, Shabana SM, Mousa SA, Mukhtar H (2014) Oral administration of naturally occurring chitosan-based nanoformulated green tea polyphenol EGCG effectively inhibits prostate cancer cell growth in a xenograft model. Carcinogenesis 35(2):415–423

Klinkesorn U, Sophanodora P, Chinachoti P, Decker EA, McClements JD (2005) Encapsulation of emulsified tuna oil in two-layered interfacial membranes prepared using electrostatic layer-by-layer deposition. Food Hydrocolloids 19(6):1044–1053
Komaiko J, McClements DJ (2015) Low-energy formation of edible nanoemulsions by spontaneous emulsification: factors influencing particle size. J Food Eng 146:122–128
Kontopidis G, Holt C, Sawyer L (2004) Invited review: beta-lactoglobulin: binding properties, structure, and function. J Dairy Sci 87(4):785–796
Lane KE, Li W, Smith C, Derbyshire E (2014) The bioavailability of an omega-3-rich algal oil is improved by nanoemulsion technology using yogurt as a food vehicle. Int J Food Sci Technol 49(5):1264–1271
Lee SH, Heng D, Ng WK, Chan HK, Tan RBH (2011) Nano spray drying: a novel method for preparing protein nanoparticles for protein therapy. Int J Pharm 403(1–2):192–200
Lee LL, Niknafs N, Hancocks RD, Norton IT (2013) Emulsification: mechanistic understanding. Trends Food Sci Technol 31(1):72–78
Levinson Y, Israeli-Lev G, Livney Y (2014) Soybean β-conglycinin nanoparticles for delivery of hydrophobic nutraceuticals. Food Biophys 9(4):332–340
Livney YD (2010) Milk proteins as vehicles for bioactives. Curr Opin Colloid Interface Sci 15(1–2):73–83
Livney YD (2012) Biopolymeric amphiphiles and their assemblies as functional food ingredient and nutraceutical delivery systems, Chapter 10. In: Garti N, McClements DJ (eds) Encapsulation technologies and delivery systems for food ingredients and nutraceuticals. Woodhead Publishing Limited, Oxford, pp 252–286
Mao Y, McClements DJ (2013) Modification of emulsion properties by heteroaggregation of oppositely charged starch-coated and protein-coated fat droplets. Food Hydrocolloids 33 (2):320–326
Markman G, Livney YD (2012) Maillard-conjugate based core–shell co-assemblies for nanoencapsulation of hydrophobic nutraceuticals in clear beverages. Food Funct 3:262–270
Matalanis A, McClements DJ (2012) Factors influencing the formation and stability of filled hydrogel particles fabricated by protein/polysaccharide phase separation and enzymatic cross-linking. Food Biophys 7(1):72–83
Matalanis A, McClements DJ (2013) Hydrogel microspheres for encapsulation of lipophilic components: optimization of fabrication & performance. Food Hydrocolloids 31(1):15–25
Matalanis A, Lesmes U, Decker EA, McClements DJ (2010) Fabrication and characterization of filled hydrogel particles based on sequential segregative and aggregative biopolymer phase separation. Food Hydrocolloids 24(8):689–701
McClements DJ (2005) Food emulsions: principles, practices, and techniques. CRC Press, Boca Raton
McClements DJ (2015) Nanoparticle- and microparticle-based delivery systems. Encapsulation, protection and release of active compounds. CRC Press, Taylor & Francis Group, LLC, Boca Raton
McClements DJ, Decker EA, Park Y, Weiss J (2009) Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. Crit Rev Food Sci Nutr 49(6):577–606
Mogol BA, Gokmen V, Shimoni E (2013) Nano-encapsulation improves thermal stability of bioactive compounds omega fatty acids and silymarin in bread. Agro Food Ind Hi-Tech 24(3):62–65
Moomand K, Lim L-T (2014) Oxidative stability of encapsulated fish oil in electrospun zein fibres. Food Res Int 62:523–532
Muller RH, Mader K, Gohla S (2000) Solid lipid nanoparticles (SLN) for controlled drug delivery —a review of the state of the art. Eur J Pharm Biopharm 50(1):161–177
Muller RH, Gohla S, Keck CM (2011) State of the art of nanocrystals–special features, production, nanotoxicology aspects and intracellular delivery. Eur J Pharm Biopharm 78(1):1–9
Mun S, Kim Y-R, McClements DJ (2015) Control of beta-carotene bioaccessibility using starch-based filled hydrogels. Food Chem 173:454–461

Nazir A, Schroën K, Boom R (2010) Premix emulsification: a review. J Membr Sci 362(1–2):1–11
Neethirajan S, Kobayashi I, Nakajima M, Wu D, Nandagopal S, Lin F (2011) Microfluidics for food, agriculture and biosystems industries. Lab Chip 11(9):1574–1586
Neo YP, Ray S, Jin J, Gizdavic-Nikolaidis M, Nieuwoudt MK, Liu D, Quek SY (2013) Encapsulation of food grade antioxidant in natural biopolymer by electrospinning technique: a physicochemical study based on zein-gallic acid system. Food Chem 136(2):1013–1021
Nisisako T (2008) Microstructured devices for preparing controlled multiple emulsions. Chem Eng Technol 31(8):1091–1098
Norton IT, Frith WJ (2001) Microstructure design in mixed biopolymer composites. Food Hydrocolloids 15(4–6):543–553
Norton JE, Gonzalez Espinosa Y, Watson RL, Spyropoulos F, Norton IT (2015) Functional food microstructures for macronutrient release and delivery. Food Funct 6(3):663–678
Oehlke K, Adamiuk M, Behsnilian D, Graef V, Mayer-Miebach E, Walz E, Greiner R (2014) Potential bioavailability enhancement of bioactive compounds using food-grade engineered nanomaterials: a review of the existing evidence. Food Funct 5(7):1341–1359
Ogawa S, Decker EA, McClements DJ (2004) Production and characterization of O/W emulsions containing droplets stabilized by lecithin-chitosan-pectin mutilayered membranes. J Agric Food Chem 52(11):3595–3600
Ostertag F, Weiss J, McClements DJ (2012) Low-energy formation of edible nanoemulsions: factors influencing droplet size produced by emulsion phase inversion. J Colloid Interface Sci 388(1):95–102
Oxley JD (2012a) Coextrusion for food ingredients and nutraceutical encapsulation: principles and technologies, Chapter 6. In: Garti N, McClements DJ (eds) Encapsulation technologies and delivery systems for food ingredients and nutraceuticals. Woodhead Publishing Limited, Oxford, Cambridge, Philadelphia, New Delhi, pp 131–149
Oxley JD (2012b) Spray cooling and spray chilling for food ingredient and nutraceutical encapsulation, Chapter 5. In: Garti N, McClements DJ (eds) Encapsulation technologies and delivery systems for food ingredients and nutraceuticals. Woodhead Publishing Limited, Oxford, Cambridge, Philadelphia, New Delhi, pp 110–130
Pan K, Zhong Q, Baek SJ (2013) Enhanced dispersibility and bioactivity of curcumin by encapsulation in casein nanocapsules. J Agric Food Chem 61(25):6036–6043
Panagiotou T, Mesite SV, Bernard JM, Chomistek KJ, Fisher RJ (2008) Production of polymer nanosuspensions using microfluidizer® processor based technologies. NSTI-Nanotech
Pandita D, Kumar S, Poonia N, Lather V (2014) Solid lipid nanoparticles enhance oral bioavailability of resveratrol, a natural polyphenol. Food Res Int 62:1165–1174
Pando D, Beltran M, Gerone I, Matos M, Pazos C (2015) Resveratrol entrapped niosomes as yoghurt additive. Food Chem 170:281–287
Paudel A, Worku ZA, Meeus J, Guns S, Van den Mooter G (2013) Manufacturing of solid dispersions of poorly water soluble drugs by spray drying: formulation and process considerations. Int J Pharm 453(1):253–284
Peltonen L, Hirvonen J (2010) Pharmaceutical nanocrystals by nanomilling: critical process parameters, particle fracturing and stabilization methods. J Pharm Pharmacol 62(11):1569–1579
Peltonen L, Valo H, Kolakovic R, Laaksonen T, Hirvonen J (2010) Electrospraying, spray drying and related techniques for production and formulation of drug nanoparticles. Expert Opin Drug Deliv 7(6):705–719
Penalva R, Esparza I, Agueeros M, Gonzalez-Navarro CJ, Gonzalez-Ferrero C, Irache JM (2015) Casein nanoparticles as carriers for the oral delivery of folic acid. Food Hydrocolloids 44:399–406
Piorkowski DT, McClements DJ (2014) Beverage emulsions: recent developments in formulation, production, and applications. Food Hydrocolloids 42:5–41
Pool H, Mendoza S, Xiao H, McClements DJ (2013) Encapsulation and release of hydrophobic bioactive components in nanoemulsion-based delivery systems: impact of physical form on quercetin bioaccessibility. Food Funct 4(1):162–174

Qian C, McClements DJ (2011) Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: factors affecting particle size. Food Hydrocolloids 25(5):1000–1008

Ramos OL, Pereira RN, Rodrigues R, Teixeira JA, Vicente AA, Xavier Malcata F (2014) Physical effects upon whey protein aggregation for nano-coating production. Food Res Int 66:344–355

Rao J, Decker EA, Xiao H, McClements DJ (2013) Nutraceutical nanoemulsions: influence of carrier oil composition (digestible versus indigestible oil) on β-carotene bioavailability. J Sci Food Agric 93(13):3175–3183

Rashidinejad A, Birch EJ, Sun-Waterhouse D, Everett DW (2014) Delivery of green tea catechin and epigallocatechin gallate in liposomes incorporated into low-fat hard cheese. Food Chem 156:176–183

Ron N, Zimet P, Bargarum J, Livney YD (2010) Beta-lactoglobulin-polysaccharide complexes as nanovehicles for hydrophobic nutraceuticals in non-fat foods and clear beverages. Int Dairy J 20(10):686–693

Saberi AH, Fang Y, McClements DJ (2013) Fabrication of vitamin E-enriched nanoemulsions by spontaneous emulsification: effect of propylene glycol and ethanol on formation, stability, and properties. Food Res Int 54(1):812–820

Saiz-Abajo M-J, Gonzalez-Ferrero C, Moreno-Ruiz A, Romo-Hualde A, Gonzalez-Navarro CJ (2013) Thermal protection of beta-carotene in re-assembled casein micelles during different processing technologies applied in food industry. Food Chem 138(2–3):1581–1587

Salminen H, Helgason T, Kristinsson B, Kristbergsson K, Weiss J (2013) Formation of solid shell nanoparticles with liquid omega-3 fatty acid core. Food Chem 141(3):2934–2943

Salvia-Trujillo L, Qian C, Martin-Belloso O, McClements DJ (2013) Modulating beta-carotene bioaccessibility by controlling oil composition and concentration in edible nanoemulsions. Food Chem 139(1–4):878–884

Schalbart P, Kawaji M (2013) Comparison of paraffin nanoemulsions prepared by low-energy emulsification method for latent heat storage. Int J Therm Sci 67:113–119

Schnettler B, Crisóstomo G, Mora M, Lobos G, Miranda H, Grunert KG (2014) Acceptance of nanotechnology applications and satisfaction with food-related life in Southern Chile. Food Sci Technol (Campinas) 34(1):157–163

Semo E, Kesselman E, Danino D, Livney YD (2007) Casein micelle as a natural nano-capsular vehicle for nutraceuticals. Food Hydrocolloids 21(5–6):936–942

Semyonov D, Ramon O, Shimoni E (2011) Using ultrasonic vacuum spray dryer to produce highly viable dry probiotics. LWT—Food Sci Technol 44(9):1844–1852

Semyonov D, Ramon O, Shoham Y, Shimoni E (2014) Enzymatically synthesized dextran nanoparticles and their use as carriers for nutraceuticals. Food Funct 5(10):2463–2474

Sengupta A, Sen Gupta S, Nandi I, Ghosh M (2015) Conjugated linolenic acid nanoparticles inhibit hypercholesterolemia induced by feeding a high-fat diet in male albino rats. J Food Sci Technol-Mysore 52(1):458–464

Shapira A, Davidson I, Avni N, Assaraf YG, Livney YD (2012) β-casein nanoparticle-based oral drug delivery system for potential treatment of gastric carcinoma: stability, target-activated release and cytotoxicity. Eur J Pharm Biopharm 80(2):298–305

Shpigelman A, Cohen Y, Livney YD (2012) Thermally-induced β-lactoglobulin-EGCG nanovehicles: loading, stability, sensory and digestive-release study. Food Hydrocolloids 29(1):57–67

Shpigelman A, Shoham Y, Israeli-Lev G, Livney YD (2014) Beta-lactoglobulin-naringenin complexes: nano-vehicles for the delivery of a hydrophobic nutraceutical. Food Hydrocolloids 40:214–224

Solans C, Solé I (2012) Nano-emulsions: formation by low-energy methods. Curr Opin Colloid Interface Sci 17(5):246–254

Soltani S, Madadlou A (2015) Gelation characteristics of the sugar beet pectin solution charged with fish oil-loaded zein nanoparticles. Food Hydrocolloids 43:664–669

Souza MP, Vaz AFM, Correia MTS, Cerqueira MA, Vicente AA, Carneiro-da-Cunha MG (2014) Quercetin-loaded lecithin/chitosan nanoparticles for functional food applications. Food Bioprocess Technol 7(4):1149–1159

Swaisgood HE (2001) Protein ingredient for carrying lipophilic nutrients. Patent number: US 6290974 B1, (North Carolina State University, USA).
Tamjidi F, Shahedi M, Varshosaz J, Nasirpour A (2013) Nanostructured lipid carriers (NLC): a potential delivery system for bioactive food molecules. Innovative Food Sci Emerg Technol 19:29–43
Tamjidi F, Shahedi M, Varshosaz J, Nasirpour A (2014) EDTA and alpha-tocopherol improve the chemical stability of astaxanthin loaded into nanostructured lipid carriers. Eur J Lipid Sci Technol 116(8):968–977
Tan C, Xue J, Lou X, Abbas S, Guan Y, Feng B, Zhang X, Xia S (2014) Liposomes as delivery systems for carotenoids: comparative studies of loading ability, storage stability and in vitro release. Food Funct 5(6):1232–1240
Ting Y, Jiang Y, Ho C-T, Huang Q (2014) Common delivery systems for enhancing in vivo bioavailability and biological efficacy of nutraceuticals. J Funct Foods 7:112–128
Tolstoguzov V (2002) Thermodynamic aspects of biopolymer functionality in biological systems, foods, and beverages. Crit Rev Biotechnol 22(2):89–174
Tolstoguzov V (2006) Phase behavior in mixed polysaccharide systems. Food Sci Technol 160:589–627 (Boca Raton, FL, United States)
Tolstoguzov V, Srinivasan D, Paraf A (1997) Protein-polysaccharide interactions. In: Food proteins and their applications. CRC Press, Boca Raton, pp 171–198
Vega C, Roos YH (2006) Invited review: spray-dried dairy and dairy-like—emulsions compositional considerations. J Dairy Sci 89(2):383–401
Vladisavljević GT, Kobayashi I, Nakajima M (2012) Production of uniform droplets using membrane, microchannel and microfluidic emulsification devices. Microfluid Nanofluid 13(1):151–178
Walker R, Decker EA, McClements DJ (2015) Development of food-grade nanoemulsions and emulsions for delivery of omega-3 fatty acids: opportunities and obstacles in the food industry. Food Funct 6(1):42–55
Walstra P (1993) Principles of emulsion formation. Chem Eng Sci 48(2):333–349
Wang QW, Allen JC, Swaisgood HE (1997) Binding of vitamin D and cholesterol to beta-lactoglobulin. J Dairy Sci 80(6):1054–1059
Weiss J, Decker EA, McClements DJ, Kristbergsson K, Helgason T, Awad T (2008) Solid lipid nanoparticles as delivery systems for bioactive food components. Food Biophys 3(2):146–154
Wooster TJ, Golding M, Sanguansri P (2008) Impact of oil type on nanoemulsion formation and Ostwald ripening stability. Langmuir 24(22):12758–12765
Xixi C, Lina Z, Shaoyun W, Pingfan R (2015) Fabrication and characterization of the nano-composite of whey protein hydrolysate chelated with calcium. Food Funct 6(3):816–823
Yang Y, McClements DJ (2013) Encapsulation of vitamin E in edible emulsions fabricated using a natural surfactant. Food Hydrocolloids 30(2):712–720
Yang J, Zhou Y, Chen L (2014a) Elaboration and characterization of barley protein nanoparticles as an oral delivery system for lipophilic bioactive compounds. Food Funct 5(1):92–101
Yang W, Xu C, Liu F, Yuan F, Gao Y (2014b) Native and thermally modified protein-polyphenol coassemblies: lactoferrin-based nanoparticles and submicrometer particles as protective vehicles for (-)-epigallocatechin-3-gallate. J Agric Food Chem 62(44):10816–10827
Yao M, Xiao H, McClements DJ (2014) Delivery of lipophilic bioactives: assembly, disassembly, and reassembly of lipid nanoparticles. Annu Rev Food Sci Technol 5(5):53–81
Yao M, McClements DJ, Xiao H (2015) Improving oral bioavailability of nutraceuticals by engineered nanoparticle-based delivery systems. Curr Opin Food Sci 2:14–19
Yi J, Lam TI, Yokoyama W, Cheng LW, Zhong F (2014) Cellular uptake of beta-carotene from protein stabilized solid lipid nanoparticles prepared by homogenization-evaporation method. J Agric Food Chem 62(5):1096–1104
Yu H, Huang Q (2013) Investigation of the cytotoxicity of food-grade nanoemulsions in Caco-2 cell monolayers and HepG2 cells. Food Chem 141(1):29–33

Zheng K, Zou A, Yang X, Liu F, Xia Q, Ye R, Mu B (2013) The effect of polymer-surfactant emulsifying agent on the formation and stability of alpha-lipoic acid loaded nanostructured lipid carriers (NLC). Food Hydrocolloids 32(1):72–78

Zhou Q-Z, Wang L-Y, Ma G-H, Su Z-G (2008) Multi-stage premix membrane emulsification for preparation of agarose microbeads with uniform size. J Membr Sci 322(1):98–104

Zimet P, Livney YD (2009) Beta-lactoglobulin and its nanocomplexes with pectin as vehicles for omega-3 polyunsaturated fatty acids. Food Hydrocolloids 23(4):1120–1126

Zimet P, Rosenberg D, Livney YD (2011) Re-assembled casein micelles and casein nanoparticles as nano-vehicles for [omega]-3 polyunsaturated fatty acids. Food Hydrocolloids 25(5):1270–1276

Chapter 6
Encapsulation Efficiency and Capacity of Bioactive Delivery Systems

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Abstract A distinctive feature of bioactive food components is their vulnerability to harsh environments and the detrimental consequences owing to their rapid degradation and inactivation. For this reason, development of food ingredients with bioactive functionalities is an on-going challenge within the global food industry. Encapsulation is one possible method to improve the stability and delivery of bioactive components or cells in food. While the last two decades have seen substantial advances in encapsulation technologies, certain constraints for the use in food need to be overcome, namely the relatively high cost the most commonly used methods, difficult scale-up and the limited array of acceptable encapsulating materials. This chapter presents an overview of the industrial need for encapsulation. Possible solutions are outlined as regards to the encapsulation materials and processing methods. The most commonly used methods are covered: immobilization cell technology, spray drying, extrusion, emulsification, fluidized bed technology and vibrating jet technique. The most widely used hydrocolloids are described with special emphasis on dairy ingredients as encapsulation systems.

6.1 Introduction

A distinctive feature of bioactive food components is their vulnerability to harsh environments and the detrimental consequences owing to their rapid degradation and inactivation. For this reason, development of food ingredients with bioactive functionalities is an ongoing challenge within the global food industry (Mattila-Sandholm

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et al. 2002). More recently, functional food innovation has increased the interest for encapsulation techniques since bioactive delivery would profit from an encapsulation procedure that protects and stabilizes bioactives during delivery to the site where adsorption is desired (Burgain et al. 2011a). With origins in the pharmaceutical industry, microencapsulation is currently utilized for controlled flavour-release and production of functional foods with bioactive compounds (Champagne and Fustier 2007). Nonetheless, encapsulation remains a specialist area in the food industry, the reason being that no universal encapsulation technique exists for all food ingredients. Design and production of microparticles and their release profiles rely upon the encapsulation technology employed and the physico-chemical properties of the active ingredient and matrix materials. Novel and traditional foods with integrated bioactives are continuously developed or improved to improve and optimize the delivery of the active ingredients; examples include fruit juice (Saarela et al. 2006), snacks (Valerio et al. 2006), frozen desserts (Di Criscio et al. 2010) and cheese (Madureira et al. 2011).

6.1.1 Defining the Need for Encapsulation

Encapsulation is a physico-chemical or mechanical process whereby bioactive components or cells are completely enveloped by a matrix for physical protection against potentially hazardous processes and adverse environmental conditions. The first factor to consider when delivering food bioactives relates to ingredient stability during storage and their incorporation directly into food to exert a desired function. Encapsulation only becomes necessary if the raw ingredient is not stable in its intended storage environment and/or the direct introduction of the bioactive into the food compromises its functionality or affects the quality of the food product used as the delivery vehicle. Once it has been established that encapsulation will offer benefits either over presentation of the ingredient in the raw format form or when its direct incorporation into food compromises a desired functionality of the ingredient, an appropriate delivery system can be designed. Furthermore, in light of the recent advances in manufacturing technologies and innovative approaches for site-specific cell delivery, bioactive encapsulation can be integrated within various processes including fermentation (Lacroix and Yidirim 2007); yoghurt manufacture (Krasaekoopt et al. 2003; Brinques and Ayub 2011); spray-drying (Muller et al. 2009) and many more. In essence, microencapsulation protects an active component (core) within a secondary material (matrix, shell, wall or encapsulant) from the surrounding environment and enables its controlled release at a target site. Microencapsulation is well established within the pharmaceutical, cosmetic, chemical and agricultural industries, but is relatively new to the food industry (Risch and Reineccius 1995). The early uses of microencapsulation in the food industry included masking undesirable flavours, controlling the release of desirable flavours, and converting liquid streams into powdered formats for convenience and improved shelf life and product yield. Encapsulation technology is increasingly being used in the food industry to protect and control delivery of food ingredients and bioactives (Champagne and Fustier 2007; Mcclements 2007; Augustin and Sanguansri 2008).

Table 6.1 Classification of the most commonly used encapsulation methods for food

Encapsulation technique	Important parameters	Advantages	Disadvantages
Extrusion (mechanical)	Diameter of the needle, flow rate, viscosity of solution, height from the cationic bath to the needle, polymer concentration, temperature	Several technologies have been developed to decrease the particle size, improve yield and homogeneity	Challenging large-scale production; Slow production yield; Limited to low-viscosity solutions
Emulsion (physico-chemical)	Stirring rate, viscosity of the solution, height from the cationic bath to the needle, polymer concentration, temperature, solubility of the polymer and solubility of the gelling agent	Easily scaled up; provides both entrapped and encapsulated core materials; small particle diameters	Small particle size but large size distribution; Relatively high operational costs due to the use of vegetable oil
Spray-drying (mechanical)	Air inlet and outlet temperature, concentration of the feed solution, polymer solubility and crystallisation form of the polymer	Low operating cost and high yield Well-established inexpensive and straightforward technology	Applied to limited polymer range; Not fitting for heat-sensitive bacteria; Produces fine powders that require high heat treatment
Fluidized-bed (mechanical)	Concentration of the feed solution, viscosity, air flow temperature and humidity	Low-cost technology Applied to a range of polymers	Difficult to achieve uniform and complete micron particles

Development of microencapsulation systems requires an understanding of the solubility and stability of the encapsulant, the physico-chemical properties of the encapsulating (matrix) materials and the processes technology used for encapsulation. Both the core and the encapsulating materials used in the formulation of encapsulated ingredients for the food industry must be of food grade. The technology to be used for encapsulation may be selected from a range of processing methods (Table 6.1), depending on the formulation and the final structure of the capsule. A number of reviews describe the history of and developments in the encapsulation of food ingredients and encapsulation technologies (Risch and Reineccius 1995; Gouin 2004; Madene et al. 2006; Augustin and Sanguansri 2008).Recent advances in encapsulation technologies have generated a major transition within the food industry from a simple manufacturing foundation to a functionality-driven food market. Encapsulation matrix materials can originate from a wide range of natural and synthetic materials; hence, the structural

arrangement of microparticles generated may be pre-determined for specific process applications. In the food industry, the concept of encapsulating a material within a defined membrane dates back to the 1930s (Shahidi and Han 1993), when capsules were produced by a complex coacervation process (Green and Schleicher 1957) known as 'locking' rather than encapsulation. Coacervation involved the dispersion of oil droplets in a mixture of two colloid materials such as gelatine, alginate or casein. Colloids must be ionisable and must exist in the mixture with opposite electric charges. This may be achieved by selection of colloid materials or by pH adjustment of the solution in which the oil droplets are dispersed in the event that one or both colloids are amphoteric. In the food industry, the principle benefits of encapsulation may be summarized as follows (Desai and Park 2005):

- Stabilization of a sensitive material from adverse interactions within the immediate environment;
- Reduction of evaporation or transfer rate of the core material to the outside environment;
- Modification of the physical characteristics of the original material to allow easier handling;
- Pulsatile, sustained or controlled release of the core material;
- Flavour or taste masking of the core material;
- Separation of reactive materials;
- Dilution of the core material when only small amounts are required.

6.1.2 Market for Encapsulated Ingredients

Consumers continue to demand high quality foods with added health benefits. The nutraceutical and functional food market is the driving force for encapsulation and delivery of bioactives to improve the quality of the finished products with added nutraceutical ingredients. Most nutraceutical ingredients are sensitive to standard food manufacturing processes, have undesirable flavours or colours, or can affect final product stability and appearance. Delivery systems that offer triggered and controlled release of the bioactive at a specific time, temperature or process are sought-after ingredients, as they offer superior functionality to formulators. As a result, global sales of encapsulated food products were approx. $20.5 billion during 2011, and this is expected to reach $36.7 billion during 2017, rising at a compound annual growth rate (CAGR) of 10.1 % (Global Industry Analysts 2010), with Asia-Pacific and North America representing the fastest-growing market. While cell protection remains a traditional function for encapsulation (Champagne et al. 2005), new encapsulation technologies, devised by academics and industrialists alike, have broadened the applications for encapsulation (Senuma et al. 2000). However, food manufacturers need to concentrate on important issues such as cost-in-use and ease of scale-up in order to generate commercially viable encapsulation techniques with broad-spectrum product applications. In general, functional food ingredients are incorporated at low levels (0.01–4.5 %); hence, the maximum cost of an encapsulation process can vary significantly (Gouin 2004).

6.2 Variation in Encapsulation

Five main structural forms of microparticles are employed for encapsulation technology as illustrated in Fig. 6.1. The most common capsule is characterized by cell entrapment within a solid matrix (Fig. 6.1a), which does not possess a distinctive membrane (Strand et al. 2004). These matrix systems are interchangeably described as capsules, microbeads (Strand et al. 2004) or microspheres (Raymond et al. 2004) and can be converted into reservoir systems consisting of single core capsules (Fig. 6.1b) by the addition of an outer shell and, if necessary, liquefaction of the core. Double- or multiple-shell microcapsules shown in Fig. 6.1c are generated by the addition of a second or multiple coating layers to the original capsule. The extra shell layers are

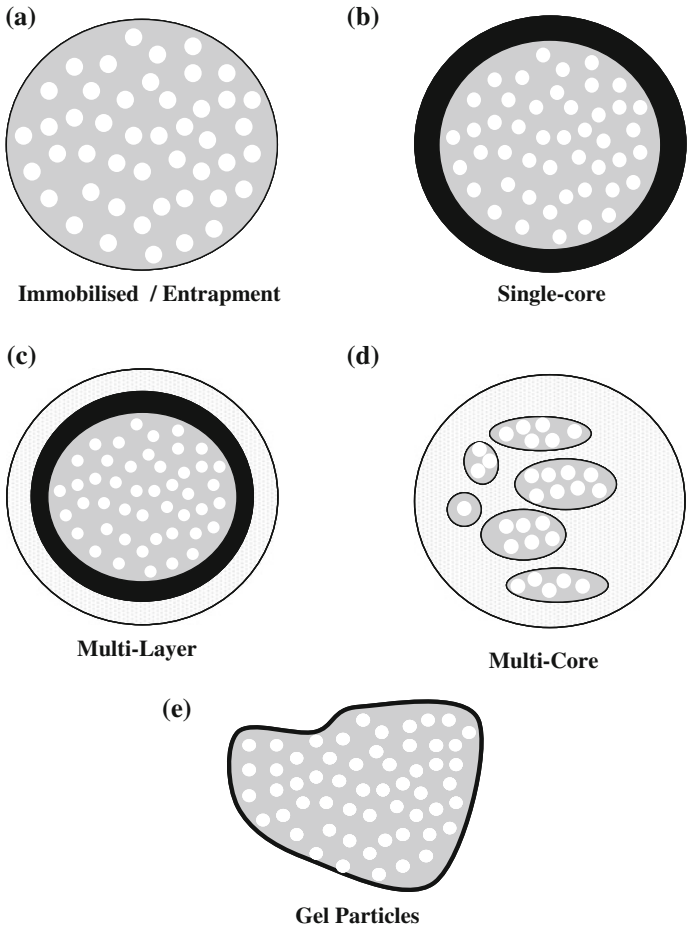

Fig. 6.1 The five main structural forms of microparticles. Types **a**, **b** can also have multiple shells added to modify stability and permeability of the capsules

added to augment the original stability/permeability characteristics of microcapsules (Heinzen et al. 2004). Multi-core capsules with two or more separate cores (Fig. 6.1d) are normally formed during emulsions (Atkin et al. 2004) while irregular-shaped gel particles can also be utilized as a general method of micro-entrapment (Fig. 6.1e). Examples of capsules with core–shell structures are conventional oil-in-water (O/W) emulsions, multiple emulsions (W/O/W), multi-layer-stabilized emulsions, solid lipid particles (O/W), nano-emulsions generated via high-pressure homogenization and/or liposomes. Hydrogels, lipospheres and biopolymeric microparticles with active cores are typical of encapsulated systems with matrix structures.

6.2.1 Function of the Matrix Material

The encapsulating materials used in food applications include proteins, carbohydrates, fats and food-grade surfactants. Dairy ingredients are food grade and readily available materials and their physico-chemical properties are highly suitable for encapsulation applications. Dairy proteins, lactose, milk fat and its natural components, and milk fat globule membrane (MFGM) components may all be utilized as encapsulating materials. Some dairy components [e.g. milk protein, peptides, hydrolysates, conjugated linoleic acid (CLA)] have been encapsulated for a specific systematic function. A wide variety of coating materials have been approved and used for functional coating in the pharmaceutical industry. These materials can be classified into four categories: (1) water-soluble polymers, (2) water-insoluble polymers, (3) water-soluble non-polymeric material and (4) water-insoluble non-polymeric material. Due to increased environmental concern and toxicity in relation to organic solvents, aqueous dispersions are preferred to organic solvent solutions for water-insoluble coating materials. The type of coating material selected can vary depending on the objective of the microencapsulation process (taste masking, enteric protection, time release, delayed release) and the desired barrier properties. By selecting an appropriate coating material, it is possible to design an encapsulation system so that it can protect the bioactive ingredient within the core, until it reaches a favourable biological environment, based on the pH, temperature, ionic strength, enzyme activity or other environmental factors. For example, in order to provide enteric protection (protection against gastric conditions in the stomach), the coatings should exhibit low permeability of the bioactive ingredient at low pH, but high permeability at high pH. This system would protect bioactives against challenging acidic environment in the stomach and provide targeted release in the lower gastrointestinal tract at higher pH.

To provide such pH-sensitive permeability, coating materials such as polymers with free carboxylic or sulphate groups should be used. At low pH, these groups are protonated and uncharged, whereas at high pH they become deprotonated, negatively charged, and therefore hydrophilic and soluble (Siepmann and Siepmann 2008). Examples of these types of polymers include proteins, fibres and carbohydrates such as carrageenans and alginates. The use of these polymers for enteric coating is reported extensively. A broad range of bioactive release profiles have further been demonstrated by varying the blend ratio of the two polymers. These techniques could be adapted for

designing a delivery vehicle for pH-sensitive bioactive ingredients. In addition, pH-sensitive coating is also useful for ensuring the stability of bioactive ingredients incorporated into food or beverages with a wide range of pH environments.

6.2.2 Thickness of the Coating Matrix

The release of bioactive ingredients from encapsulation systems is mainly governed by diffusion through the coating/matrix layer. Therefore, as the coating thickness increases, the release rate will be slower as a result of the longer diffusion path (Lecomte et al. 2003; Huyghebaert et al.2005a, b, c). It is often necessary to apply a greater amount of coating material to achieve the desired protection, when applying the coating material in the form of an aqueous dispersion compared to applying it in an organic solvent solution (Thoma and Bechtold 1999). The relative size of the encapsulation core has an influence on the thickness of coating required in order to achieve similar release profiles. Rahman et al. (2005) demonstrated that when two batches of core pellets with different mesh sizes but with narrow size distributions were coated under the same conditions, a significant difference in the release profiles was observed. The larger size pellets showed a significantly slower release rate when compared with the smaller pellets. It is clear that smaller pellets need more coating to establish a similar release profile. Siepmann et al. (2006b) demonstrated that microfilm structures are created at different coating levels. The lower number of polymer particle layers in thin films illustrates higher probability of continuous water-filled porous channels within the coating film connecting the core to the surrounding fluid. In contrast, in thicker films there are a higher number of polymer particle layers, and even with the same degree of coalescence, the probability for the existence of continuous water-filled channels directly connecting the inner and outer surfaces of the film coatings is much lower. Therefore, depending on the coating level (thickness), the release of the core ingredient can be primarily controlled by diffusion through water-filled channels, or by diffusion through the macromolecular network for an incomplete coalesced film. The former will lead to a high release rate and the latter to much lower release rates in the same surrounding fluid conditions.

6.2.3 Matrix Release Properties

By adjusting the internal structure and properties of the coating or the matrix material, the release profile of the bioactive core can be manipulated. An understanding of the physico-chemical properties of the encapsulating matrix, how it interacts with the core, and its behaviour during processing is essential in order to develop an encapsulation system. The bioactive ingredient must be protected up to the appropriate time; a so-called trigger needs to initiate the controlled release. Triggers for the release of the core can be changes in the pH, temperature, mechanical shear or degradation of the

matrix material (e.g. by enzymes). Application of control upon the core release in response to an appropriate trigger is strongly influenced by (1) the chosen encapsulant material; (2) the ratio and formulation; and (3) the processing technique used to generate an encapsulated ingredient (Ubbink and Krüger 2006).

Various studies have been reported on the use of different polymers for controlled release of encapsulated ingredients, such as cellulose-based enteric polymers (Varshosaz et al. 1997; Marvola et al. 1999), lipids (Siepmann et al. 2006a) and dairy protein (Doherty et al. 2011). Depending on the product application, an additional layer of protective shell coating can be applied to the surface of the matrix systems, to further deter/delay the release of the encapsulated ingredient. Krogars et al. (2000) developed a delivery system for targeted release to the distal part of the small intestine and the colon, by preparing film-coated matrix pellets in which enteric polymer was used both as a binder in the pellets and also as a coating material. Although coated beads are widely used in the pharmaceutical industry, the process is often complex, time consuming and costly, and may not be a suitable option for the food industry. Due to the ease of manufacture and robust nature of the release properties, matrix systems may be preferred to typical pharmaceutical-based (reservoir) systems (Varma et al. 2004).

The underlying release mechanisms from a matrix system are more complex, as they can involve simultaneous structural modifications to encapsulated bioactive ingredients and microencapsulating polymers (Grassi et al. 2010). Due to the fundamental differences in the physico-chemical properties of bioactive ingredients and coating polymers used, the dominating chemical reaction and/or physical mass transfer processes can differ significantly from system to system. The release of the bioactive ingredient can be governed by swelling and erosion of the matrix, dissolution and diffusion through the matrix, or by a combination of both (Varma et al. 2004; Grassi et al. 2010). When the hydrophobic matrix is exposed to aqueous fluid, the eluting medium penetrates the matrix through the pores present in the matrix and the bioactive ingredients diffuse out of the matrix via the liquid-filled porous network (Varma et al. 2004). According to the theory of Heller and Baker (Siepmann and Peppas 2001), the dissolution of the encapsulated ingredients from water-insoluble polymers is first controlled by diffusion through the polymer. The diffusion release rate decreases with time due to the increasing diffusion path length. After a certain time period, this effect is overcompensated by the increasing permeability due to progressive erosion of the polymer. Polymer erosion may be due to hydrolytic backbone cleavage, resulting in the polymer chains being converted into small, water-soluble molecules.

The release rate of bioactives normally decreases initially, and then increases as a function of time. For poorly soluble bioactives commonly found in pharmaceutical applications, dissolution and diffusion is practically non-existent. In the case of poorly soluble or non-soluble bioactives, the release is primarily by an erosion mechanism (Varma et al. 2004). As erosion continues, the effective diffusion of the bioactive will decrease; therefore, the release rate will increase as a function of time. Erosion kinetics strongly depend on polymer characteristics, i.e. functional groups from which it is built, the type of monomers, co-polymerization and neighbouring groups (Siepmann and Peppas 2001). Erosion can be classified into two different kinds, namely surface

(heterogeneous) erosion and bulk (homogeneous) erosion (Grassi et al. 2010). In the first scenario, only the periphery sections of the matrix are impacted, however in the second scenario, erosion also impacts the polymeric bulk phase. For the effects of surface erosion, polymer degradation is much faster than water intrusion into the polymer bulk. Therefore, degradation occurs mainly in the peripheral polymer layers. On the contrary, bulk erosion polymer degradation is much slower than water uptake by the system. Therefore, the encapsulation system is rapidly hydrated and polymer chains are cleaved throughout the system (Grassi et al. 2010). The type of erosion that occurs depends on the hydrophobicity and morphology of the matrix material/polymer. Surface erosion is more common with hydrophobic polymers; however, hydrophilic polymers are utilized for bulk erosion effects (Pothakamury and Barbosa-Cánovas 1995). The release rate and release mechanism from an encapsulation system primarily depends on the solubility of the bioactive to be encapsulated. Highly aqueous, soluble bioactives show faster release rates, while poorly water-soluble ingredients (<0.01 mg/ml) often result in incomplete release due to poor solubility and low dissolution rates for the matrix (Varma et al. 2004). For insoluble bioactives, erosion will be governed by the kinetics of release. When the matrix contains a water-soluble bioactive ingredient, an appropriate combination of diffusion and erosion mechanisms determine the release kinetics.

6.3 Processing Techniques

In addition to the formulations, different processing techniques will result in matrix systems with different physical and mechanical properties. These differences can relate to changes in bioactive ingredient release behaviour. The nomenclature of encapsulation methodologies extends from extrusion and emulsification to spray drying and fluidized-bed as summarized in Table 6.1. Additional techniques such as liposome, co-crystallisation and molecular inclusion can generate microparticles, but their use is limited by cost constraints (Champagne and Kailasapathy 2008). The creation of novel encapsulation techniques can originate from practical insights into other fields since almost any non-food process can be transformed into food-grade encapsulation technology. Hence, for the purpose of this review, the most commonly used techniques will be presented.

6.3.1 Immobilization Cell Technology (ICT)

Immobilization cell technology (ICT) is a universal design strategy for micro-entrapment (Anal and Singh 2007), whereby bioactive cells are immobilized or entrapped randomly within/throughout a continuous matrix, which is often a hydrogel. ICT was originally introduced to enhance the efficiency of biotechnology production processes, since matrix properties permit rapid and efficient separation of producer cells and undesirable metabolites. ICT is successfully employed in a

wide spectrum of disciplines within the food industry inclusive of fermentation processes (De Giulio et al. 2005). Starter culture and lactic acid production are imperative requirements during the manufacture of fermented dairy products and the failure of starter culture performance will inevitably generate an abundance of adverse effects on quality, appearance, texture and flavour of the end product. Prévost and Divies (1987, 1988) performed the first dairy application of ICT by separate cell entrapment of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in calcium alginate gel beads for continuous inoculation of milk for yoghurt production. Incorporation of ICT for continuous yoghurt manufacture represents a complex procedure. Advantages include elevated cell and lactic acid concentrations with better control of residence time, acidity and bacilli/cocci ratios. Hence, ICT is a practical approach or for the delivery of bioactive cells.

6.3.2 Spray Drying

Spray-drying is a quintessential preservation and encapsulation method for bioactive compounds and functional bioactives alike (Riveros et al. 2009). The development of spray-drying equipment and techniques evolved over several decades and came to prominence during the second world war due to the requirement to reduce transport weight of foodstuffs (Patel et al. 2009). The speed of drying and continuous production capabilities were useful for drying large amounts of bioactive compounds and cultures. Since then, much research has been reported on the spray-drying of bioactives without loss of activity in order to overcome the difficulties involved in handling and maintaining product inventory. Gum arabic and starches represent customary support materials for spray drying since they form spherical microparticles during the drying process. The merits of this process have ensured its dominance, particularly in the dairy industry to concentrate surplus supplies of milk and milk products into dry stable powders with a long shelf life due to low moisture content (<4 %), which inhibits microbial development during storage. Spray drying is initiated with the atomization of a liquid feedstock (solution, suspension, dispersion or emulsion) into a spray of droplets. The spray is produced by either rotary (wheel) or nozzle atomizers, and upon contact with hot air in a drying chamber, these droplets lose their moisture rapidly (Fig. 6.2a). Evaporation of moisture from the droplets and the formation of dry particles proceed under controlled temperature and airflow conditions. Powder is continuously discharged from the drying chamber and recovered from the exhaust gases using a cyclone or a bag filter by centrifugal action, whereby dense powder particles are forced towards the cyclone walls while the lighter, moist air is directed away through the exhaust pipes (Fig. 6.2). The dried product can be in the form of powders, granules or agglomerates, depending on the physical and chemical properties of the feed, the dryer design and final powder properties desired. The FILTERMAT® Spray Dryer is frequently used in food and dairy applications due to its low outlet temperature and subsequent high thermal efficiency (Patel et al. 2009).

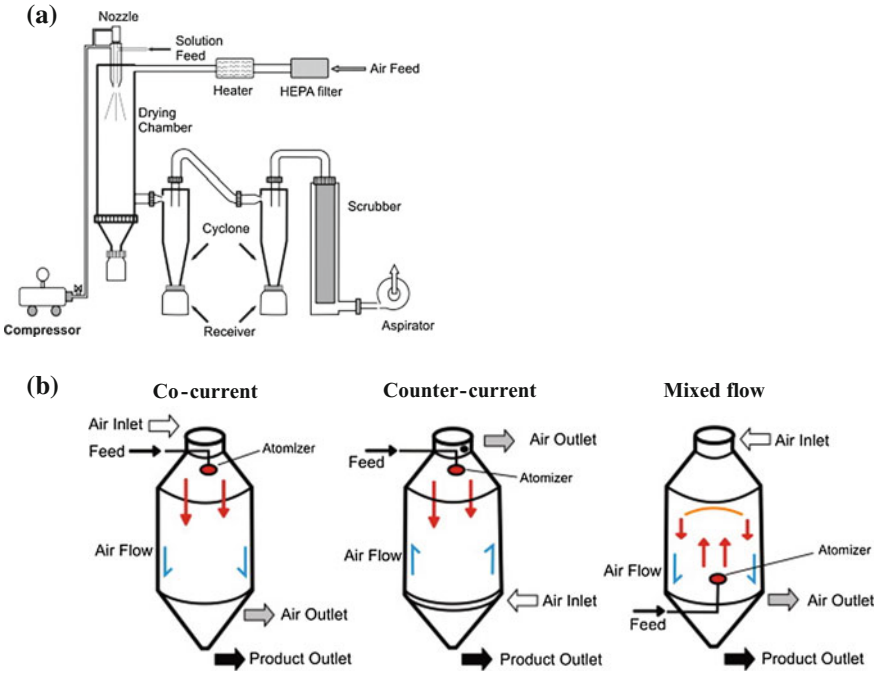

Fig. 6.2 Typical spray drying operation utilizing a centrifugal atomizer (**a**) and a cyclone separator [adopted from Devakate et al. (2009)] and typical air flow patterns (**b**)

Typical product-air flow patterns in spray dryers are shown in Fig. 6.2b (Vega-Mercado et al. 2001): co-current, counter-current and mixed flow. In the co-current process, the droplets and air pass through the dryer in the same direction. The droplets meet the air at the highest temperature. This causes rapid surface evaporation, while it is still wet, providing safe conditions for heat-sensitive materials. This approach is commonly utilized for microencapsulation of antibiotics, peptides and protein since thermal exposure is minimized and product recovery occurs 15 °C below the outlet temperature (Wan et al. 1992). The counter-current configuration sprays the droplets in the opposite direction to the hot air flow, which exposes the dry product to high temperatures. This design can only be used for non-heat-sensitive materials and is less commonly used than the co-current configuration. Mixed flow is a combination of co-current and counter-current flow patterns. A nozzle is positioned in the bottom of the chamber, forcing the spray to travel upward until overcome by gravity and the downward flow of the drying medium.

Spray-drying is highly reproducible, easily scaled up and suitable for industrial applications. The residence time required for drying particles is short (<30 s) and most of the wet particles experience only the wet bulb temperature during removal of the majority of water; therefore, heat damage to dry materials is generally only slight. Despite popularity, however, maintenance of cell viability during spray

drying of heat-sensitive cultures remains an industrial challenge with additional process restrictions based on the limited selection of shell materials with appropriate solubility properties (Weissbrodt and Kunz 2007). The process of spray-drying creates many technical problems for bioactives, inclusive of heat, osmotic stress, ribosome/DNA damage and loss of membrane integrity, which is affiliated with lipid and protein damage (Brennan et al. 1986; Teixeira et al. 1997). Thus, an alternative encapsulation technology may be required for guaranteed bioavailability of heat-sensitive bioactives.

6.3.3 Extrusion

Extrusion is a mechanical matrix-based technique for the production of microspheres/microcapsules for biotechnological and food applications. Essentially, bioactive materials can be dispersed or encapsulated in a solid matrix—either hydrophobic or hydrophilic—in order to speed up or reduce the release kinetics, depending on the final therapeutic target. In essence, desired microbead droplets are generated by extruding a hydrocolloid solution through a jet orifice at high pressure using mechanical means (cutting or vibrational forces) to increase the customary dripping process at the orifice. Droplets free-fall into a solution with immediate solidification either by physical (cooling or heating) or chemical means such as gelation. The droplet surface will solidify almost upon immediate contact with the gelling bath, and droplets will be fully gelled after prolonged gelation time, which entraps cells in a three-dimensional hydrocolloid lattice. The size and shape of the microbeads depends on the diameter of the orifice and distance of free-fall, which dictates subsequent dispersibility in the final product. Such a method known as 'droplet extrusion' was explored by Ouwerx et al. (1998), who investigated the physical and chemical parameters influencing the behaviour of alginate beads prepared by dropping aqueous sodium alginate into various divalent cations, resulting in large beads of 2.4–3.0 mm in size. Extrusion on the other hand, characterized by both single and multiple channel systems, has successfully produced microspheres of much smaller sizes (Freitas et al. 2005).

Alginate is the most commonly employed material for the generation of hydrocolloid beads by extrusion technology (Doleyres and Lacroix 2005). Alginate provides a gentle environment for entrapped material, low cost-in-use, matrix biocompatibility (Krasaekoopt et al. 2003) and no requirement for high temperature, pressure or complex instrumentation. Alginates, composed of alternating block of 1-4 linked α-L-guluronic acid and β-D-mannuronic acid residues, are water-soluble linear polysaccharides extracted from brown algae. Alginate gelation can be achieved by reaction with divalent cations such as Ca^{2+} which bind to the α-L-guluronic acid blocks in a highly cooperative manner (Smidsrod and Skjak-Braek 1990). As a result, each alginate chain dimerizes to form junctions with many other chains, resulting in gel network formation (Dupuy et al. 1994). However, diffusion of low molecular weight bioactives through semi-permeable gel networks such as

alginate represent a major issue in relation to encapsulation efficiency resulting in unsatisfactory acid resistance and bioactive function. These limitations compromise the feasibility of extrusion with consequent motivation to discover alternative gelling agents. Several hydrocolloids are actively employed for extrusion (Table 6.2), including kappa-carrageenan (Krasaekoopt et al. 2003), gellan gum (Sun and Griffiths 2000) and combinations thereof (Sultana et al. 2000).

Depending on the application, an additional protective layer of shell coating was applied to these extrusion systems, with chitosan preferably utilized as a coating material for targeted delivery to the colon (Chávarri et al. 2010). However, this approach may not be a viable option for food manufacturers due to the costly, time-consuming nature of the process. Owing to their desirable gelation function, dairy proteins are highlighted for simple extrusion methodologies (Doherty et al. 2011, 2012), with Hébrard et al. (2010) demonstrating the benefits of whey protein coating for enhanced bioactive function while Heidebach et al. (2009b) exploited the functionality of casein proteins for enhanced bioactive cell survival. Due to fundamental differences in the physico-chemical properties of bioactive ingredients and polymer materials, the release mechanism in matrix systems may involve dissolution or diffusion through the matrix, swelling and erosion of the matrix or a combination of both (Grassi et al. 2010; Varma et al. 2004).

Table 6.2 Hydrocolloids commonly employed for extrusion

Gelling agent	Conditions of gelation	References
Alginate	Sets in the presence of divalent counter-ions	Chandramouli et al. (2004)
Carrageenan (ι, κ, λ)	Microparticles generated by in situ ionic gelation	Belyaeva et al. (2004), Ellis and Jacquier (2009)
Gum arabic	Highly branched structure with proteinaceous material capable of stabilizing oil-in-water (O/W) emulsions	Lian et al. (2003), Hsiao et al. (2004), Desmond et al. (2002)
Milk proteins	Structural and physiochemical properties facilitate their functionality as compatible delivery agents	Reid et al. (2007), Guerin et al. (2003), Heidebach et al. (2009a), Livney (2010), Doherty et al. (2011)
Xanthan gum	Utilized as a rheological agent and stabilizer in O/W emulsions	Papagianni and Anastasiadou (2009)
Pectin	Gels at low pH with divalent ions and interacts with positively charged protein molecules	Sandoval-Castilla et al. (2010), Madziva et al. (2006)
Starch	Modified starches exhibit good spray-drying properties, and resistant starch is an ideal surface for probiotic adherence and targeted intestinal delivery	O'Riordan et al. (2001), Kailasapathy and Chin (2000)
Gelatine	Thermo-reversible gels form on cooling	Annan et al. (2008), Hyndman (1993)

6.3.4 Emulsification

Emulsification consists of at least two immiscible liquids—usually oil or water—with one of the liquids being dispersed as small spherical droplets in the other. Emulsion can be classified according to the spatial arrangement of the oil and water phases whereby a small volume of aqueous cell-polymer suspension (discontinuous phase) is added to a large volume of an oil (continuous phase). The bioactive mixture is homogenized (Fig. 6.3) to form a water-in-oil (W/O) emulsion, and once the water-in-oil emulsion is formed, the water-soluble polymer must be insolubilized (cross-linked) to form gel particles within the oil phase (Burgain et al. 2011b). Conversely, a system involving oil droplets dispersed in an aqueous phase is called an oil-in-water emulsion (O/W), and the size of the internal phase in both emulsion systems will determine the final microparticle properties and the support material utilized will further dictate the choice of insolubilization method. Typically, the diameter of the droplets in food systems range from 0.1 to 100 μm.

The emulsion encapsulation technique was developed by Nilsson et al. (1983) as a universal method for immobilization of sensitive cells and currently represents the predominant technique for bioactive encapsulation in batch and continuous processes (Audet et al. 1990; Lacroix et al. 1990). Emulsion technology is generally applied for the encapsulation of bioactives in aqueous solutions, which can either be used directly or in the liquid state or can be dried to form a powder (i.e. by spray, roller or freeze-drying) after emulsification. Gel beads can be introduced into a second polymer solution to create a coating layer for additional bioactive protection or enhanced organoleptic properties in products including ice-cream (Homayouni et al. 2008). The technique is easily scaled-up and generates high bioactive functionality (Chen and Chen 2007); however, particles have large size distributions and operational costs are relatively high due to the inclusion of vegetable oil and emulsifiers such as Tween 80. It is critical, however, to highlight the fact that food emulsions must initially be stable since emulsions are kinetically rather than

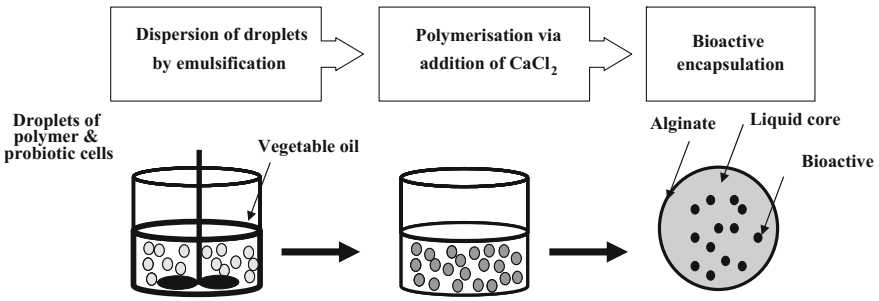

Fig. 6.3 Schematic of the emulsification procedure involving a small volume of probiotic-polymer suspension (i.e. the discontinuous phase) added to a large volume of vegetable oil (i.e. the continuous phase). After homogenization, water-in-oil (W/O) emulsion is formed and the water-soluble polymer is gelled within the oil phase

thermodynamically stable two-phase systems with inevitable separation of oil and water phases. Hence, when considering food emulsions as a delivery mechanism for sensitive ingredients two issues must be addressed: (1) the necessity to stabilise the emulsion against separation prior to delivery/consumption and (2) control to prevent emulsion destabilization to achieve the required release under appropriate physiological conditions. Emulsions may be stabilized by combining process and formulation designs including homogenization, which facilitates droplet break-up and the creation of a dispersed phase.

6.3.5 Fluidized Bed Technology

Fluid spray coating is another technique utilized for protection of bioactives. This process involves a three-step procedure governed by suspension and circulation of particles using hot air or gas, and it represents an efficient approach for the application of uniform layers of shell material onto solid particles. A liquid coating material is sprayed through a nozzle onto the particles to initiate film formation in the coating chamber, where the hot atmosphere facilitates rapid evaporation during the coating procedure. The core material (bioactive) needs to be in a solid form and remain in motion in a specially designed vessel, either by injection of air at the bottom or by a rotary action (Champagne and Fustier 2007). A succession of wetting and drying stages is initiated for optimum film formation, which involves coalescence and fusion of polymer particles on the core material surface. Poor adhesion between the coating and the surface of the core material may affect stability of the encapsulated component due to the accumulation of moisture at the core-coating interface, which may further compromise mechanical protection of encapsulation. There are three different types of fluidized-bed coaters characterized by the position of the nozzle: top spray, bottom spray ('Wurster' coating) and tangential spray with rotating disk (rotor pellet coating). Ease of technology scale-up promotes fluidized spray coating as a popular methodology for bioactive encapsulation in neutraceutical products, and it is also adaptable for multi-layer coating procedures. This technology provides the high energy needed to evaporate water, which offers an ideal application for aqueous-based coatings including proteins (Dewettinck and Huyghebaert 1999) and coating particles which tend to agglomerate when wet (Williams III and Liu 2006). Relative to available technologies, fluidized bed endorses appreciably more versatile controlled-release potential due to its capacity to coat particles with basically any kind of polymer, and operational costs are comparable or slightly lower than spray-drying (Muller et al. 2009). Despite the potential advances of fluidized bed coating in bioactive applications, most information for the technology is proprietary with widespread industrial integration impeded by high cost-in-use. Integration of encapsulation technologies is also a viable possibility whereby fluidized bed technology is used to agglomerate fine (10–100 μm) spray-dried particles to impart better protection, functionality and shelf life (Buffo and Reineccius 2001).

6.3.6 *Vibrating Jet Technique*

The vibrating jet technique is primarily performed by liquid extrusion through a vibrating jet nozzle (Del Gaudio et al. 2005), which is the main recognized commercial technique for conversion of fluid dispersions into micro-beads/capsules (Senuma et al. 2000). The method is based on the principle of laminar-jet breakup using vibrational frequency with defined amplitude to an extruded jet flow (Fig. 6.4). In brief, when a liquid is extruded through a nozzle at certain flow rates, it produces a laminar jet, which can break up freely into short lengths by sporadic disturbances. These segments then form spherical droplets due to the force of surface tension. However, natural breakup is unpredictable, resulting in micro-beads with large size distributions.

The application of a permanent sinusoidal force at defined frequencies to the jet will generate one droplet per hertz of applied frequency (Rayleigh 1879). Figure 6.4a shows a schematic representation of a lab-scale encapsulator that can generate single core microbeads; production of multicore capsules using a concentric nozzle. Physical properties of the microbeads and bioactive release properties depend on various parameters inclusive of nozzle diameter, flow rate of the laminar jet, size of the frequency at defined amplitude, and viscosity of the extruded liquid (Fig. 6.5).

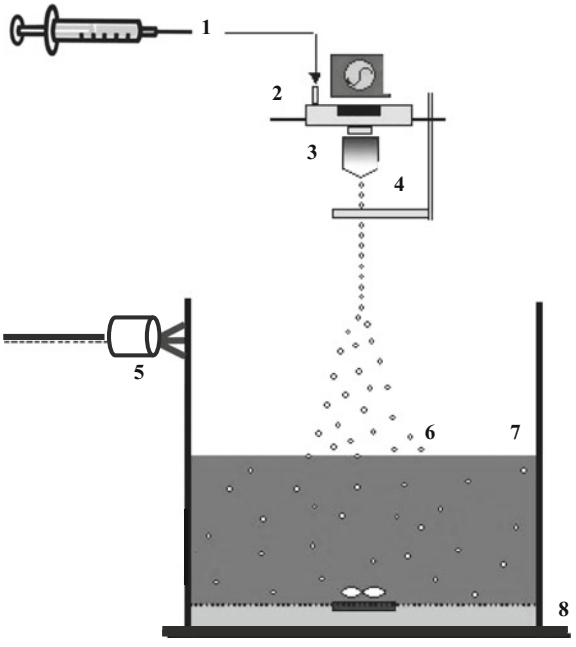

Fig. 6.4 Inotech® Encapsulator is illustrated, with numbers indicating the flow process during the extrusion procedure: *1* feed line; *2* vibrating nozzle; *3* nozzle (50–1000 μm); *4* electrode; *5* stroboscope lamp; *6* jet stream 'drop zone'; *7* outer acetate stream; *8* temperature control and agitation

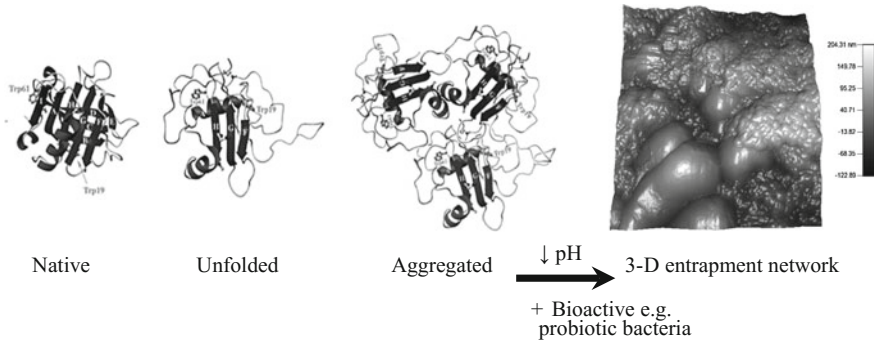

Fig. 6.5 Schematics of globular protein unfolding during the cold gelation with subsequent addition of bioactive compounds/cells. The atomic force microscope (AFM) image reveals the progressive engulfment of individual particles by processed milk protein (Doherty et al. 2011)

6.4 Dairy Encapsulation Systems

6.4.1 Dairy Proteins

The diverse functionalities of milk proteins have been used for millennia. Protein fractions with specific functions are widely available and are important building blocks for product innovation, including encapsulation (Livney 2010). Bovine milk proteins contain approximately 13 % solids, consisting of 4 % fat, 3 % protein, 4 % lactose, 1 % ash and other minor components (Fox and McSweeney 2003). The protein content can be divided into two categories: those precipitating upon adjustment of milk to pH 4.6, caseins; and those remaining soluble at pH 4.6, whey proteins. There are four caseins found in milk, α_{s1}-, α_{s2}-, β- and κ-casein. In milk, these caseins form coarse colloidal micelles in conjunction with metal ions. These micelles have a molecular weight of $\sim 10^8$ Da and are between 60 and 500 nm in diameter (Fox and Brodkorb 2008). The whey proteins, which comprise β-lactoglobulin, α-lactalbumin, immunoglobulins, bovine serum albumin and other minor proteins, are found at a concentration of about 0.7 % in milk. For many years, whey proteins were considered waste products of cheese making and casein production industries. However, increased industry impetus to reduce waste from manufacturing processes provided whey proteins with the necessary recognition as a profitable ingredient with nutritional, functional and gelation properties suitable for encapsulation (Reid et al. 2007; Heidebach et al. 2009b).

The formation of cold-set whey protein gels proved successful for protection and delivery of bioactives (Hebrard et al. 2006) and probiotic bacteria (Reid et al. 2007), with further applications for enhanced retinol (Beaulieu et al. 2002) and nutrient (Remondetto et al. 2004) and bioavailability. Remondetto et al. (2002) exploited the cold-set gelation mechanism by adding ferrous salt to β-lactoglobulin solutions to address global mineral deficiency concerns. Filamentous and particulate gels were

produced depending on the iron/protein ratio. The filamentous form is created by linear aggregation of structural units maintained by hydrophobic interactions, whereas particulate forms are produced by random aggregation of structural units controlled by van der Waals forces.

These microstructures revealed different sensitivities to environmental conditions such as pH, and digestive enzymes with filamentous gels illustrating potential as matrices for iron transport and absorption (Remondetto et al. 2004). Lee and Rosenberg (2000) developed a microencapsulation technology based on heat denaturation of whey proteins for the generation of an O/W/O double emulsion producing microcapsules with an extremely smooth and uniform outer surface. This morphological feature contrasts remarkably with traditional spray-drying microcapsules, where water evaporation causes collapse of the microcapsule and lower encapsulation efficiency.

Whey protein emulsions have also attracted interest in food formulations since their flexibility and amphiphilic nature permit rapid absorption to the emulsion interface where they self-aggregate and form continuous and homogenous membranes around oil droplets through intermolecular β-sheet interactions (Lefevre and Subirade 2003). Chen et al. (2006) demonstrated α-tocopherol protection under gastrointestinal conditions using cold-set β-lactoglobulin emulsion gels where molecular delivery was regulated by gel biodegradation. Hence, composite systems such as protein emulsions may broaden functional food development with novel organoleptic properties (Mcclements 2007). Beaulieu et al. (2002) generated microbeads with diameters ranging from 1 to 2 mm, based on the principle of emulsification with subsequent Ca^{2+}-induced gelation of pre-denatured whey protein. Results confirmed the ability of whey protein beads to protect bioactive molecules from gastric pH conditions for targeted intestinal absorption. Furthermore, whey proteins microspheres were also produced via emulsification/internal cold gelation (Chen and Subirade 2005). The size distribution of these protein structures, homogenous with alginate matrices, ranged from 3 to 10 μm using a method based on the release of calcium ions from an acid-soluble calcium salt in emulsified pre-denatured protein solution. This approach favours the production of small protein microbeads in large quantities via control of emulsification conditions for integration into various food formulations with a targeted intestinal release mechanism. Meanwhile, casein proteins have also been utilized as soluble sodium-caseinate or calcium-caseinate for encapsulation (Heidebach et al. 2009a, 2010) and green tea polyphenols (Dehkharghanian et al. 2009) by means of acid gelation.

6.4.2 Protein Hydrolysates

Controlled proteolysis of α-lactalbumin (α-LA) results in the formation of self-assembled α-LA nanotubes (approx. 110 × 20 nm) via association of the protein hydrolysates in the presence of Ca ions (Graveland-Bikker et al. 2006; Ipsen

and Otte 2007). The inner cavity of the tubes (8 nm diameter) is a putative site for encapsulation of molecules such as vitamins, minerals or enzymes. Furthermore, the nanotubes are heat stable (72 °C, 40 s) and able to withstand the freeze-drying process. The stability of the nanotubes may be further enhanced by cross-linking (e.g. using transglutaminase). The nanotubes can also be fitted with lipid caps that open and close to allow controlled release of the encapsulated bioactives. Milk protein hydrolysates can also be used as encapsulants in emulsion-based systems. Numerous studies have shown that proteolysis of the milk proteins modifies their functionality, including their surface-active and gelation properties. The effect of proteolysis on the physical functionality of proteins depends on the type of milk protein and the hydrolysis conditions (Foegeding et al. 2002; Kilara and Panyam 2003). For example, Chobert et al. (1987, 1988a, b) showed that limited hydrolysis of casein (2–10 %) could increase its emulsifying activity. Euston et al. (2001) showed hydrolysis (10–27 %) improved the emulsifying capacity of WPC (Whey Protein Concentrate) generally containing between 30 to 80 % protein. Treatment of whey proteins with a protease has also been shown to induce gel formation more rapidly during heat treatment compared to the intact proteins (Chen et al. 1994). Gel properties can also be manipulated by modulating hydrolysis conditions (Otte et al. 1996). These properties allow milk protein hydrolysates to function as effective encapsulants and may lead to the development of unique delivery system.

6.4.3 Milk Fat Delivery Systems

The major component of milk fat is triglycerides (98 %). The composition of milk fat is highly complex, with over 400 fatty acids and 200 triglycerides identified (Gresti et al. 1993). Milk fat melts over the temperature range of −40 to +40 °C. The melting temperature of milk fat is a function of the crystalline state (Walstra and Jenness 1984) and the composition of the triglycerides. The fractionation process provides milk fat with various triglyceride compositions and is performed to differentiate the physical and functional properties of milk fat, such as crystallization behaviour and melting point (Kaylegian and Lindsay 1995). In the area of encapsulation, milk fat fractions with a defined melting point can be exploited; for example, to facilitate finer control over the release of core materials that have been embedded in a fat matrix.

Milk fat is an obvious carrier for lipophilic compounds. Braun and Olson (1986) encapsulated proteins and peptides in milk fat and investigated their functionality. Butter oil capsules demonstrated good freeze–thaw dispersibility. However, when the capsules were held at high incubation temperatures (>32 °C), such as those that may be encountered during cheese making, the microcapsules showed poor temperature stability.

Active cores may also be embedded in a solid fat matrix. A high-melting point fraction of milk fat was used to encapsulate (48 % encapsulation efficiency) protease enzymes (Kailasapathy and Lam 2005), which were subsequently

incorporated (73.5 %) into cheese curd. Encapsulation of the proteases accelerated cheese ripening compared to the control cheese. Jackson and Lee (1991) prepared lipid-coated microcapsules of iron to fortify cheese. However, neither a high melting point fraction of milk fat nor hydrogenated milk fat was a suitable coating for this application.

Milk fat can also be used in emulsion-based systems. Encapsulation of α-tocopherol in a stearin-rich milk fat fraction was prepared as a caseinate O/W emulsion to protect the antioxidant against degradation during storage (Relkin et al. 2008). Al-Nabulsi et al. (2006) microencapsulated lactoferrin in a polyglycerol condensed ricinoleate (PGPR)-stabilized butterfat-corn oil W/O emulsion. The fat encapsulant improved the antimicrobial activity of lactoferrin in cured meat by protecting it against adverse environmental conditions.

6.4.4 Milk Fat Globule Membrane as an Encapsulant

The Milk Fat Globule Membrane (MFGM) is a complex mixture of glycolipids, phospholipids and proteins (Singh 2006). MFGM phospholipids constitute 26–31 % of its total lipid content, and together with proteins constitute more than 90 % of the membrane dry weight (Singh 2006). It has a high nutritional value (Spitzberg 2005; Dewettincka et al. 2008) and acts as a natural barrier against lipolysis of milk fat in milk. MFGM material is a natural emulsifier and behaves differently from milk proteins (caseins, β-LG) when used for stabilizing emulsions. Corredig and Dalgleish (Corredig and Dalgleish 1998a, b) revealed that MFGM-stabilized O/W emulsions are less susceptible to interfacial displacement by low molecular weight surfactants than milk protein-stabilized emulsions, and are not affected by the presence of other milk proteins (caseins, whey). These properties have been attributed to a strong interfacial interaction between MFGM and the oil. The phospholipids may also contribute by lowering the interfacial tension. However, the source of the raw MFGM, the isolation procedure and the conditions during emulsion formation are critical, in order to maximise MFGM functionality (Singh 2006).

Liposomes prepared from MFGM phospholipids possess several advantages over their soy phospholipid counterparts, including a thicker membrane, lower membrane permeability and higher phase transition temperature (Thompson and Singh 2006). These properties afford the MFGM-derived phospholipid liposomes with improved environmental stability (e.g. heat, pH, presence of divalent cations) compared to liposomes prepared from soy-derived phospholipids. Furthermore, in vivo studies show MFGM enhances intestinal drug absorption (Liu et al. 1995), indicating opportunities for the use of MFGM for bioactive delivery.

6.5 Conclusion and Perspectives

It is important to remember that encapsulation requires a multi-disciplinary approach. Consideration of the core/bioactive and the effects of the encapsulant, formulation, process design and process conditions are all crucial to achieve the optimum result for a defined objective of protection. In this respect, it is anticipated that a greater focus will be given to enhancing our understanding of the effect of core materials on encapsulation processes and encapsulant properties. Knowledge that provides fundamental information on the nature of the interaction between the bioactives and encapsulation matrices in order to anticipate release properties will be required to formulate and design delivery systems for a greater range of bioactive materials. It is highly likely that more functional foods in the future will contain colloids with complex structures, not only to control bioactive release, but also with increasingly complicated structures designed to impact on human health. It can be foreseen that with new strategies for stabilization of sensitive bioactives and development of novel approaches to site-specific carrier targeting, proteins, encapsulation will may play a role for increasing the efficacy of functional foods over the next decade. However, at the present stage, greater fundamental understanding of interactions on the molecular level and their impact on functional properties of bioactives is still required to bridge the gap to efficacious bioactive commercial applications.

References

Al-Nabulsi AA, Han JH, Liu Z, Rodrigues-Vieira ET, Holley RA (2006) Temperature-sensitive microcapsules containing lactoferrin and their action against *Carnobacterium viridans* on Bologna. J Food Sci 71(6):M208–M214

Anal AK, Singh H (2007) Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends Food Sci Technol 18:240–251

Annan NT, Borza AD, Hansen LT (2008) Encapsulation in alginate-coated gelatin microspheres improves survival of the probiotic Bifidobacterium adolescentis 15703T during exposure to simulated gastro-intestinal conditions. Food Res Int 41(2):184–193

Atkin R, Davies P, Hardy J, Vincent B (2004) Preparation of aqueous core/polymer shell microcapsules by internal phase separation. Macromolecules 37:7979–7985

Audet P, Paquin C, Lacroix C (1990) Batch fermentation with a mixed culture of lactic acid bacteria immobilized separately in kappa-carrageenan locust bean gum gel beads. Appl Microbiol Biotechnol 32(6):662–668

Augustin M, Sanguansri L (2008) Encapsulation of bioactives. In: Food materials science. Springer, pp 577–601

Beaulieu L, Savoie L, Paquin P, Subirade M (2002) Elaboration and characterization of whey protein beads by an emulsification/cold gelation process: application for the protection of retinol. Biomacromolecules 3(2):239–248

Belyaeva E, Valle DD, Poncelet D (2004) Immobilization of α-chymotrypsin in κ-carrageenan beads prepared with the static mixer. Enzyme Microb Technol 34:108–113

Braun SD, Olson NF (1986) Encapsulation of proteins and peptides in milk fat. Encapsulation efficiency and temperature and freezing stability. J Microencapsul 3(2):115–126

Brennan M, Wanismail B, Johnson M, Ray B (1986) Cellular damage in dried Lactobacillus acidophilus. J Food Prot (USA)

Brinques GB, Ayub MAZ (2011) Effect of microencapsulation on survival of *Lactobacillus plantarum* in simulated gastrointestinal conditions, refrigeration, and yogurt. J Food Eng 103 (2):123–128. doi:10.1016/j.jfoodeng.2010.10.006

Buffo RA, Reineccius GA (2001) Comparison among assorted drying processes for the encapsulation of flavors. Perfumer Flavorist 26:58–67

Burgain J, Gaiani C, Linder M, Scher J (2011a) Encapsulation of probiotic living cells: from laboratory scale to industrial applications. J Food Eng 104(4):467–483. doi:10.1016/j.jfoodeng.2010.12.031

Burgain J, Gaiani C, Linder M, Scher J (2011b) Encapsulation of probiotic living cells: techniques and industrial applications in the food field. J Food Eng 104(4):467–483

Champagne CP, Fustier P (2007) Microencapsulation for the improved delivery of bioactive compounds into foods. Curr Opin Biotechnol 18(2):184–190

Champagne CP, Kailasapathy K (2008) Encapsulation of probiotics. In: Garti N (ed) Delivery and controlled release of bioactives in foods and nutraceuticals. Woodhead publishing Ltd., Cambridge, pp 344–369

Champagne CP, Gardner NJ, Roy D (2005) Challenges in the addition of probiotic cultures to foods. Crit Rev Food Sci Nutr 45:61–84

Chandramouli V, Kailasapathy K, Peiris P, Jones M (2004) An improved method of microencapsulation and its evaluation to protect Lactobacillus spp. in simulated gastric conditions. J Microbiol Methods 56(1):27–35

Chávarri M, Marañón I, Ares R, Ibáñez FC, Marzo F, Villarán MdC (2010) Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions. Int J Food Microbiol 142(1):185–189

Chen MJ, Chen KN (2007) Applications of probiotic encapsulation in dairy products. In: Lakkis JM (ed) Encapsulation and controlled release technologies in food systems. Wiley-Blackwell, USA, pp 83–107

Chen L, Subirade M (2005) Preparation of algiante-whey protein microspheres by emulsification/internal gelation and their application in healthy food. Paper presented at the conference on bioencapsulation Kingston

Chen SX, Swaisgood HE, Foegeding EA (1994) Gelation of beta.-lactoglobulin treated with limited proteolysis by immobilized trypsin. J Agric Food Chem 42(2):234–239

Chen LY, Remondetto GE, Subirade M (2006) Food protein-based materials as nutraceutical delivery systems. Trends Food Sci Technol 17(5):272–283

Chobert J-M, Sitohy M, Whitaker JR (1987) Specific limited hydrolysis and phosphorylation of food proteins for improvement of functional and nutritional properties. J Am Oil Chem Soc 64(12):1704–1711

Chobert JM, Bertrand-Harb C, Nicolas MG (1988a) Solubility and emulsifying properties of caseins and whey proteins modified enzymically by trypsin. J Agric Food Chem 36(5):883–892

Chobert JM, Sitohy MZ, Whitaker JR (1988b) Solubility and emulsifying properties of caseins modified enzymatically by *Staphylococcus aureus* V8 protease. J Agric Food Chem 36(1):220–224

Corredig M, Dalgleish DG (1998a) Buttermilk properties in emulsions with soybean oil as affected by fat globule membrane-derived proteins. J Food Sci 63:476–480

Corredig M, Dalgleish DG (1998b) Effect of heating of cream on the properties of milk fat globule membrane isolates. J Agric Food Chem 46:2533–2540

De Giulio B, Orlando P, Barba G, Coppola R, De Rosa M, Sada A, De Prisco PP, Nazzaro F (2005) Use of alginate and cryo-protective sugars to improve the viability of lactic acid bacteria after freezing and freeze-drying. World J Microbiol Biotechnol 29:739–746

Dehkharghanian M, Lacroix M, Vijayalakshmi MA (2009) Antioxidant properties of green tea polyphenols encapsulated in caseinate beads. Dairy Sci Technol 89:485–499

Del Gaudio P, Colombo P, Colombo G, Russo P, Sonvico F (2005) Mechanisms of formation and disintegration of alginate beads obtained by prilling. Int J Pharm 302:1–9

Desai KGH, Park HJ (2005) Recent developments in microencapsulation of food ingredients. Drying Technol 23:1361–1394

Desmond C, Ross RP, O'Callaghan E, Fitzgerald G, Stanton C (2002) Improved survival of *Lactobacillus paracasei* NFBC 338 in spray-dried powders containing gum acacia. J Appl Microbiol 93(6):1003–1011

Devakate RV, Patil VV, Waje SS, Thorat BN (2009) Purification and drying of bromelain. Sep Purif Technol 64:259–264

Dewettinck K, Huyghebaert A (1999) Fluidized bed coating in food technology. Trends Food Sci Technol 10:163–168

Dewettincka K, Rombauta R, Thienpontan N, Lea TT, Messensb K, Van Camp J (2008) Nutritional and technological aspects of milk fat globule membrane material. Int Dairy J 18 (5):436–457

Di Criscio T, Fratianni A, Mignogna R, Cinquanta L, Coppola R, Sorrentino E, Panfili G (2010) Production of functional probiotic, prebiotic, and synbiotic ice creams. J Dairy Sci 93 (10):4555–4564. doi:10.3168/jds.2010-3355

Doherty SB, Gee VL, Ross RP, Stanton C, Fitzgerald GF, Brodkorb A (2011) Development and characterisation of whey protein micro-beads as potential matrices for probiotic protection. Food Hydrocolloids 25(6):1604–1617

Doherty SB, Auty MA, Stanton C, Ross RP, Fitzgerald GF, Brodkorb A (2012) Application of whey protein micro-bead coatings for enhanced strength and probiotic protection during fruit juice storage and gastric incubation. J Microencapsul 29(8):713–728. doi:10.3109/02652048.2011.638994

Doleyres Y, Lacroix C (2005) Technologies with free and immobilised cells for probiotic bifidobacteria production and protection. Int Dairy J 15(10):973–988

Dupuy B, Arien A, Minnot AP (1994) FT-IR of membranes made with alginate/polylysine complexes. Variations with the mannuronic or guluronic content of the polysaccharides. Artif Cells Blood Substit Biotechnol 22(1):71–82

Ellis A, Jacquier JC (2009) Manufacture of food grade κ-carrageenan microspheres. J Food Eng 94 (3–4):316–320

Euston SR, Finnigan SR, Hirst RL (2001) Heat-induced destabilization of oil-in-water emulsions formed from hydrolyzed whey protein. J Agric Food Chem 49(11):5576–5583

Foegeding EA, Davis JP, Doucet D, McGuffey MK (2002) Advances in modifying and understanding whey protein functionality. Trends Food Sci Technol 13(5):151–159

Fox PF, Brodkorb A (2008) The casein micelle: historical aspects, current concepts and significance. Int Dairy J 18(7):677–684

Fox PF, McSweeney PLH (2003) Advanced dairy chemistry, vol Proteins Part A, 3rd edn. Kluwer Academic/Plenum, New York

Freitas S, Merkle HP, Gander B (2005) Microencapsulation by solvent extraction/evaporation: reviewing the state of the art of microsphere preparation process technology. J Controlled Release 102(2):313–332

Global Industry Analysts (2010) Food encapsulation: a global strategic business report. San Jose, California

Gouin S (2004) Microencapsulation: industrial appraisal of existing technologies and trends. Trends Food Sci Technol 15(7–8):330–347. doi:10.1016/j.tifs.2003.10.005

Grassi M, Grassi G, Lapasin R, Colombo I (2010) Understanding drug release and absorption mechanisms: a physical and mathematical approach. CRC Press, Boca Raton

Graveland-Bikker JF, Schaap IA, Schmidt CF, de Kruif CG (2006) Structural and mechanical study of a self-assembling protein nanotube. Nano Lett 6(4):616–621

Green BK, Schleicher L (1957) Oil-containing microscopic capsules and method of making them USA Patent

Gresti JM, Bogaut CM, Bezard J (1993) Functional properties of butter oil made from bovine milk with experimentally altered fat composition. J Dairy Sci 90:5018–5030

Guerin D, Vuillemard JC, Subirade M (2003) Protection of bifidobacteria encapsulated in polysaccharide-protein gel beads against gastric juice and bile. J Food Prot 66(11):2076–2084

Hebrard G, Blanquet S, Beyssac E, Remondetto G, Subirade M, Alric M (2006) Use of whey protein beads as a new carrier system for recombinant yeasts in human digestive tract. J Biotechnol 127(1):151–160

Hébrard G, Hoffart V, Beyssac E, Cardot JM, Alric M, Subirade M (2010) Coated whey protein/alginate microparticles as oral controlled delivery systems for probiotic yeast. J Microencapsul 27(4):292–302

Heidebach T, Först P, Kulozik U (2009a) Microencapsulation of probiotic cells by means of rennet-gelation of milk proteins. Food Hydrocolloids 23(7):1670–1677

Heidebach T, Först P, Kulozik U (2009b) Transglutaminase-induced caseinate gelation for the microencapsulation of probiotic cells. Int Dairy J 19(2):77–84

Heidebach T, Först P, Kulozik U (2010) Influence of casein-based microencapsulation on freeze-drying and storage of probiotic cells. J Food Eng 98(3):309–316

Heinzen C, Berger A, Marison IW (2004) Use of vibration technology for jet break-up for encapsulation of cells and liquids in monodisperse microcapsules. In: Nedovic V, Willaert R (eds) Fundamentals of cell immobilisation technology. Kluwer Academic Publishers, Dordrecht

Homayouni A, Azizi A, Ehsani MR, Yarmand MS, Razavi SH (2008) Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. Food Chem 111(1):50–55. doi:10.1016/j.foodchem.2008.03.036

Hsiao HC, Lian WC, Chou CC (2004) Effect of packaging conditions and temperature on viability of microencapsulated bifidobacteria during storage. J Sci Food Agric 84(2):134–139

Huyghebaert N, Vermeire A, Neirynck S, Steidler L, Remaut E, Remon JP (2005a) Development of an enteric-coated formulation containing freeze-dried, viable recombinant *Lactococcus lactis* for the ileal mucosal delivery of human interleukin-10. Eur J Pharm Biopharm 60 (3):349–359. doi:10.1016/j.ejpb.2005.02.012

Huyghebaert N, Vermeire A, Remon JP (2005b) In vitro evaluation of coating polymers for enteric coating and human ileal targeting. Int J Pharm 298(1):26–37. doi:10.1016/j.ijpharm.2005.03.032

Huyghebaert N, Vermeire A, Rottiers P, Remaut E, Remon JP (2005c) Development of an enteric-coated, layered multi-particulate formulation for ileal delivery of viable recombinant *Lactococcus lactis*. Eur J Pharm Biopharm 61(3):134–141. doi:10.1016/j.ejpb.2005.04.002

Hyndman C (1993) Microencapsulation of *Lactococcus-lactis* within cross-linked gelatin membranes. J Chem Technol Biotechnol (1986) 56(3):259

Ipsen R, Otte J (2007) Self-assembly of partially hydrolysed α-lactalbumin. Biotechnol Adv 25(6):602–605

Jackson LS, Lee K (1991) Microencapsulated iron food fortification. J Food Sci 56:1047–1050

Kailasapathy K, Chin J (2000) Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and Bifidobacterium spp. Immunol Cell Biol 78(1):80–88

Kailasapathy K, Lam SH (2005) Application of encapsulated enzymes to accelerate cheese ripening. Int Dairy J 15:929–939

Kaylegian KE, Lindsay RC (1995) Milk fat usage and modification. In: Handbook of milk fat fractionation technology and application. AOCS Press, Champaign

Kilara A, Panyam D (2003) Peptides from milk proteins and their properties. Crit Rev Food Sci Nutr 43(6):607–633

Krasaekoopt W, Bhandari B, Deeth H (2003) Evaluation of encapsulation techniques of probiotics for yoghurt. Int Dairy J 13(1):3–13

Krogars K, Heinamaki J, Vesalahti J, Marvola M, Antikainen O, Yliruusi J (2000) Extrusion–spheronization of pH-sensitive polymeric matrix pellets for possible colonic drug delivery. Int J Pharm 199:187–194

Lacroix C, Yidirim S (2007) Fermentation technologies for the production of probiotics with high viability and functionality. Curr Opin Biotechnol 18(2):176–183. doi:10.1016/j.copbio.2007.02.002

Lacroix C, Paquin C, Arnaud JP (1990) Batch fermentation with entrapped growing-cells of lactobacillus casei—optimization of the rheological properties of the entrapment gel matrix. Appl Microbiol Biotechnol 32(4):403–408

Lecomte F, Siepmann J, Walther M, MacRae RJ, Bodmeier R (2003) Blends of enteric and GIT-insoluble polymers used for film coating: physicochemical characterization and drug release patterns. J Controlled Release 89(3):457–471

Lee SJ, Rosenberg M (2000) Whey protein-based microcapsules prepared by double emulsification and heat gelation. Lebensmittel-Wissenschaft und Technologie 33:80–88

Lefevre T, Subirade M (2003) Formation of intermolecular beta-sheet structures: a phenomenon relevant to protein film structure at oil-water interfaces of emulsions. J Colloid Interface Sci 263(1):59–67

Lian WC, Hsiao HC, Chou CC (2003) Viability of microencapsulated bifidobacteria in simulated gastric juice and bile solution. Int J Food Microbiol 86(3):293–301. doi:10.1016/s0168-1605(02)00563-9

Liu H-X, Adachi I, Horikoshi I, Ueno M (1995) Mechanism of promotion of lymphatic drug absorption by milk fat globule membrane. Int J Pharm 118(1):55–64

Livney YD (2010) Milk proteins as vehicles for bioactives. Curr Opin Colloid Interface Sci 15(1–2):73–83. doi:10.1016/j.cocis.2009.11.002

Madene A, Jacquot M, Scher J, Desobry S (2006) Flavour encapsulation and controlled release–a review. Int J Food Sci Technol 41(1):1–21

Madureira AR, Brandao T, Gomes AM, Pintado ME, Malcata FX (2011) Technological optimization of manufacture of probiotic whey cheese matrices. J Food Sci 76(2):E203–E211. doi:10.1111/j.1750-3841.2010.02008.x

Madziva H, Kailasapathy K, Philips M (2006) Evaluation of alginate–pectin capsules in Cheddar cheese as a food carrier for the delivery of folic acid. LWT—Food Sci Technol 39(2):146–151

Marvola M, Nykanen P, Rautio S, Isonen N, Autere AM (1999) Enteric polymers as binders and coating materials in multiple-unit site specific drug delivery systems. Eur J Pharm Sci 7:259–267

Mattila-Sandholm T, Myllarinen P, Crittenden R, Mogensen G, Fonden R, Saarela M (2002) Technological challenges for future probiotic foods. Int Dairy J 12(2–3):173–182

Mcclements DJ (2007) Critical review of techniques and methodologies for characterization of emulsion stability. Crit Rev Food Sci Nutr 47(7):611–649

Muller JA, Ross RP, Fitzgerald GF, Stanton C (2009) Manufacture of probiotic bacteria. In: Charalampopoulos D, Rastall RA (eds) Prebiotics and probiotics science and technology. Springer, New York, pp 725–759. doi:10.1007/978-0-387-79058-9_18

Nilsson H, Silvegren C, Tornell B (1983) A calorimetric investigation of suspension and emulsion polymerization of vinyl-chloride. Angew Makromol Chem 112:125–142

O'Riordan K, Andrews D, Buckle K, Conway P (2001) Evaluation of microencapsulation of a Bifidobacterium strain with starch as an approach to prolonging viability during storage. J Appl Microbiol 91(6):1059–1066

Otte J, Ju Z, Faergemand M, Lomholt S, Qvist K (1996) Protease-induced aggregation and gelation of whey proteins. J Food Sci 61(5):911–916

Ouwerx C, Velings N, Mestdagh M, Axelos M (1998) Physico-chemical properties and rheology of alginate gel beads formed with various divalent cations. Polym Gels Networks 6(5):393–408

Papagianni M, Anastasiadou S (2009) Encapsulation of *Pediococcus acidilactici* cells in corn and olive oil microcapsules emulsified by peptides and stabilized with xanthan in oil-in-water emulsions: studies on cell viability under gastro-intestinal simulating conditions. Enzyme Microb Technol 45(6–7):514–522

Patel R, Patel M, Suthar A (2009) Spray drying technology: an overview. Indian J Sci Technol 2(10):44–47

Pothakamury UR, Barbosa-Cánovas GV (1995) Fundamental aspects of controlled release in foods. Trends Food Sci Technol 6(12):397–406

Prévost H, Divies C (1987) Fresh fermented cheese production with continuous pre-fermented milk by a mixed culture of mesophilic lactic streptococci entrapped in Ca-Alginate. Biotechnol Lett 9(11):789–794

Prévost H, Divies C (1988) Continuous pre-fermentation of milk by entrapped yoghurt bacteria. I. Development of the process. Milchwissenschaft 43(10):621–625

Rahman MA, Kwon NH, Won MS, Choe ES, Shim YB (2005) Functionalized conducting polymer as an enzyme-immobilizing substrate: an amperometric glutamate microbiosensor for in vivo measurements. Anal Chem 77(15):4854–4860. doi:10.1021/ac050558v

Rayleigh FRS (1879) On the capillary phenomena of jets. Proc London Math Soc 10:71–97

Raymond MC, Neufeld RJ, Poncelet D (2004) Encapsulation of brewers yeast in chitosan coated carrageenan microspheres by emulsification/thermal gelation. Artif Cells Blood Substit Biotechnol 32:275–291

Reid AA, Champagne CP, Gardner N, Fustier P, Vuillemard JC (2007) Survival in food systems of *Lactobacillus rhamnosus* R011 microentrapped in whey protein gel particles. J Food Sci 72(1): M031–M037

Relkin P, Yung J, M, Kalnin D, Ollivon M (2008) Structural behaviour of lipid droplets in protein-stabilized nano-emulsions and stability of α-tocopherol. Food Biophys 3:163–168

Remondetto GE, Paquin P, Subirade M (2002) Cold gelation of B-Lactoglobulin in the presence of iron. J Food Sci 67:586–595

Remondetto GE, Beyssac E, Subirade M (2004) Iron availability from whey protein hydrogels: an in vitro study. J Agric Food Chem 52(26):8137–8143

Risch SJ, Reineccius G (1995) Encapsulation and controlled release of food ingredients. In: ACS symposium series, USA

Riveros B, Ferrer J, Borquez R (2009) Spray drying of a vaginal probiotic strain of *Lactobacillus acidophilus*. Drying Technol 27(1):123–132

Saarela M, Virkajarvi I, Nohynek L, Vaari A, Matto J (2006) Fibres as carriers for *Lactobacillus rhamnosus* during freeze-drying and storage in apple juice and chocolate-coated breakfast cereals. Int J Food Microbiol 112(2):171–178. doi:10.1016/j.ijfoodmicro.2006.06.019

Sandoval-Castilla O, Lobato-Calleros C, García-Galindo HS, Alvarez-Ramírez J, Vernon-Carter EJ (2010) Textural properties of alginate–pectin beads and survivability of entrapped Lb. casei in simulated gastrointestinal conditions and in yoghurt. Food Res Int 43(1):111–117

Senuma Y, Lowe C, Zweifel Y, Hilborn JG, Marison I (2000) Alginate hydrogel microspheres and microcapsules prepared by spinning disk atomization. Biotechnol Bioeng 67:616–622

Shahidi F, Han XQ (1993) Encapsulation of food ingredients. Crit Rev Food Sci Nutr 33:501–547

Siepmann J, Peppas NA (2001) Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Adv Drug Deliv Rev 48(2–3):139–157

Siepmann J, Siepmann F (2008) Mathematical modeling of drug delivery. Int J Pharm 364(2): 328–343. doi:10.1016/j.ijpharm.2008.09.004

Siepmann F, Muschert S, Flament MP, Leterme P, Gayot A, Siepmann J (2006a) Controlled drug release from Gelucire-based matrix pellets: experiment and theory. Int J Pharm 317(2):136–143. doi:10.1016/j.ijpharm.2006.03.006

Siepmann F, Siepmann J, Walther M, MacRae R, Bodmeier R (2006b) Aqueous HPMCAS coatings: effects of formulation and processing parameters on drug release and mass transport mechanisms. Eur J Pharm Biopharm 63(3):262–269. doi:10.1016/j.ejpb.2005.12.009

Singh N (2006) The milk fat globule membrane—a biophysical system for food applications. Curr Opin Colloid Interface Sci 11:154–163

Smidsrod O, Skjak-Braek G (1990) Alginate as immobilization matrix for cells. Trends Biotechnol 8(3):71–78

Spitzberg V (2005) Bovine milk fat globule membrane as a potential nutraceutical. J Dairy Sci 88:2289–2294

Strand BL, Skjakbraek G, Gaserod O (2004) Microcapsule formulation and formation. In: Nedovic V, Willaert R (eds) Fundamentals of cell immobilisation biotechnology. Kluwer Academic Publishers, Dordrecht

Sultana K, Godward G, Reynolds N, Arumugaswamy R, Peiris P, Kailasapathy K (2000) Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. Int J Food Microbiol 62(1–2):47–55

Sun W, Griffiths MW (2000) Survival of bifidobacteria in yogurt and simulated gastric juice following immobilization in gellan-xanthan beads. Int J Food Microbiol 61(1):17–25

Teixeira P, Castro H, Mohácsi-Farkas C, Kirby R (1997) Identification of sites of injury in *Lactobacillus bulgaricus* during heat stress. J Appl Microbiol 83(2):219–226

Thoma K, Bechtold K (1999) Influence of aqueous coatings on the stability of enteric coated pellets and tablets. Eur J Pharm Biopharm 47(1):39–50

Thompson A, Singh H (2006) Preparation of liposomes from milk fat globule membrane phospholipids using a microfluidizer. J Dairy Sci 89(2):410–419

Ubbink J, Krüger J (2006) Physical approaches for the delivery of active ingredients in foods. Trends Food Sci Technol 17(5):244–254

Valerio F, De Bellis P, Lonigro SL, Morelli L, Visconti A, Lavermicocca P (2006) In vitro and in vivo survival and transit tolerance of potentially probiotic strains carried by artichokes in the gastrointestinal tract. Appl Environ Microbiol 72(4):3042–3045. doi:10.1128/aem.72.4.3042-3045.2006

Varma MV, Kaushal AM, Garg A, Garg S (2004) Factors affecting mechanism and kinetics of drug release from matrix-based oral controlled drug delivery systems. Am J Drug Deliv 2(1):43–57

Varshosaz J, Kenned RA, Gipps EM (1997) Effect of binder level and granulating liquid on phenylbutazone pellets prepared by extrusion spheronization. Drug Dev Ind Pharm 23(6):611–618

Vega-Mercado H, Gongora-Nieto MM, Barbosa-Cánovas GV (2001) Advances in dehydration of foods. J Food Eng 49(4):271–289

Walstra P, Jenness R (1984) Dairy chemistry and physics. Wiley, New York

Wan LS, Heng PW, Chia CG (1992) Spray drying as a process for microencapsulation and the effect of different coating polymers. Drug Dev Ind Pharm 18(9):997–1011

Weissbrodt J, Kunz B (2007) Influence of hydrocolloid interactions on their encapsulation properties using spray-drying. Minerva Biotecnol 19(1):27

Williams RO III, Liu J (2006) Influence of processing and curing conditions on beads coated with an aqueous dispersion of cellulose acetate phthalate. Eur J Pharm Biopharm 49:243–252

Chapter 7
β-Lactoglobulin-Based Nano and Microparticulate Systems for the Protection and Delivery of Bioactives

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Abstract The new paradigms in human nutrition and the ever-increasing consumer demand for safe and healthy food products have encouraged research in functional foods and nutraceuticals as pharmaceutical surrogates. Food proteins are abundant and from renewable sources, with functional groups conferring interesting structural and functional properties. Their ability to bind small ligands and to form aggregates and electrostatic complexes with other food macromolecules provides numerous applications for oral delivery technology. The current review focuses on the major milk protein β-lactoglobulin, its techno-functional properties and its applications in the formulation of nano- and micro-sized oral delivery platforms.

7.1 Introduction

Active compounds such as probiotics, bioactive peptides, antioxidants and vitamins with physiological benefits beyond basic nutritional functions such as reducing the risk of chronic disease, are often referred to as nutraceuticals. In Canada, they

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fall under the regulation of natural health products (NHPs), which went into effect on January 1, 2004 (Canada 2013). The market for NHPs is increasing rapidly worldwide. The market value is estimated at around US $117 billion from the three most important sources alone—the Unites States, Japan and Western Europe (Núñez Sellés 2011). This increases to between $300 and $400 billion when other areas such as Eastern Europe, Latin America, Asia or Africa are considered.

The development of the ideal oral system for the protection and delivery of NHPs has become a focus of tremendous research worldwide, particularly in the food and pharmaceutical sciences. The popularity of oral delivery systems has soared, and sales were already expected to reach over $67 billion a few years ago (Kim and Pack 2006; Ranade and Cannon 2011). The designation 'oral delivery system' usually implies 'controlled delivery' of bioactive compounds, which involves the efficiency of delivery and the enhancement of solubility, absorption, bioavailability, safety and duration of action (Ranade and Cannon 2011). In addition to increasing patient comfort and compliance, oral delivery systems can achieve sustained, controlled and site-specific release of bioactives (Santus and Baker 2003; Kim and Pack 2006; Ranade and Cannon 2011; Murray 2011). Consequently, from a pharmaceutical point of view, serum concentrations are less prone to fluctuations, and toxicity and dosing frequency are reduced (Ranade and Cannon 2011). In the food industry, delivery systems are used as carriers for functional ingredients, protecting those ingredients from hostile environmental factors including heat, light, oxygen, high pressure and shear forces during processing, storage and utilization, until the intact bioactive ingredient is delivered to the target site; the list of advantages is not exhaustive. The release rate of the bioactive molecule can then be modulated via the control of conditions including temperature, ionic strength, pH and digestive enzymes (Weiss et al. 2006).

The release kinetics from the oral delivery system rely on the chemical composition and/or the structure of the carrier system. In general, mechanisms of release such as diffusion result from physical (erosion) or chemical decomposition (degradation); swelling and osmotic pressure lead to an expansion of the carrier system, thus expelling the bioactive out of the carrier matrix (Ranade and Cannon 2011; Murray 2011). The chemical composition and structure of the oral delivery system are of utmost importance in achieving effective release of bioactives. With the size of oral delivery systems decreasing dramatically, concerns have been raised regarding the toxicity of such carriers and the resulting degradation products. Indeed, reduced size may lead to an increase in toxicity, given that small sized carriers might reach and accumulate in regions within cells or tissue inaccessible to macroscopic particles of the same composition. In fact, the size of oral delivery systems has decreased from capsules, tablets or powder forms to microparticles, nanoparticles and nanocomplexes. Microparticles are less than about 1000 μm while nanoparticles and nanocomplexes are less than 100 nm in size (Chen et al. 2006; Weiss et al. 2006). Therefore, biopolymers which are biocompatible and biodegradable with negligible to null toxicity are increasingly used to fabricate oral delivery systems. Milk whey protein fits well with these criteria, and thus whey protein-based delivery systems have been the focus of countless peer-reviewed

articles (Beaulieu et al. 2002; Chen and Subirade 2005; Chen et al. 2006; Chen and Subirade 2006; Hebrard et al. 2006; Hebrard et al. 2009; Hebrard et al. 2010; Hebrard et al. 2013). However, whey proteins are composed of diverse proteins with different structures, properties and functions, making it difficult to distinguish the role and degree of participation of each protein in the formation of the carrier system (O'Regan et al. 2009). However, most functional properties of whey isolates, including aggregates and gel formation, are attributed to one major protein, β-lactoglobulin (βlg) (Boland 2011; Nicolai et al. 2011). The present review therefore concentrates on the major whey protein β-lactoglobulin (βlg) and its functionality as a micro and nano-based oral delivery system.

7.2 Structure and Functionality of βlg

The structure and amino acid composition of βlg have been the focus of extensive research (Oliveira et al. 2001; Kontopidis et al. 2004; Loch et al. 2011; Sawyer 2013). Thus, only the structural characteristics and physicochemical properties related to bioactive protection and delivery are detailed in the present review.

Well defined crystal structures indicate that βlg is a globular protein of the lipocalin family, with a molecular weight of 18.3 kDa and an isoelectric point (p*I*) of 5.3 (Oliveira et al. 2001; Boland 2011; Sawyer 2013). The two main genetic variants of βlg, A and B, differ from a mutation occurring at amino acid sequence position 64 ($Asp_A \rightarrow Gly_B$) and 118 ($Val_A \rightarrow Ala_B$). The overall conformation of the molecule remains fairly the same, although the substitutions lead to dissimilarities in properties such as heat stability and resistance to pressure denaturation (Botelho et al. 2000; Oliveira et al. 2001). The information presented in the current review will refer to βlg variant B.

The protein is constituted of 162 amino acids, including all 20 amino acids in relative amounts that make it exceptional and valuable nutritionally. In fact, compared to theoretical common values computed for proteins, βlg comprises about 17 % more essential amino acids (Mehra and O'Kennedy 2009). The tertiary structure of βlg is composed of 8 % of α-helix, 45 % of β -sheet and 47 % of random coil (Loch et al. 2011; Oliveira et al. 2001; Sawyer 2013). βlg has an overall radius of 2 nm, with almost 90 % of its mass within 1 nm of the surface and nearly 60 % within 0.5 nm (Liang and Subirade 2012). The structure of the monomer of βlg is composed of nine strands of antiparallel β-sheets (strands A to I), eight (strands A to H) of which wrap around to form a flattened, conical barrel, also called the central calyx. The cylindrical-shaped calyx has a length of 15 Å, with hydrophobic walls composed of two sheets made of strands A–D and strands E–H. Strand A (residues 16–27) participates in both sheets due to its 90º bend at its midpoint (Ser21), while the ninth strand (I) extends the EFGHA sheet. The neighboring strands within the sheets are connected via a loop (Uhrínová et al. 2000). The dimer interface is formed in part by strand I and the loop connecting strand A to B. Strand A is preceded by a three-turn α-helix while another one lies in the A–B loop. The structure of βlg

contains two disulfide bridges consisting of Cys66–Cys160, connecting the C–D loop to the carboxyl-terminal region, and Cys106–Cys119 links strands G and H. An additional Cys at position 121 is buried and thus remains free. At pH values from 5 to about 7, including that of the milk (∼pH 6.6), βlg exists as a dimer in solution; below pH 3 and above 7, the monomeric conformation predominates (Uhrínová et al. 2000; Sawyer 2013). Upon modification of the pH, βlg undergoes different structural transitions categorized into distinct classes (M, Q, N and R), and their interrelation is as follows: M ↔ Q ↔ N ↔ R (Sakurai and Goto 2007).

Reports indicate that moderate to considerable pH-induced structural transitions of βlg occur between pH 2.5 and 8, while above pH 9, βlg undergoes significant and irreversible structural modification, also called base-induced unfolding of the protein (Uhrínová et al. 2000; Taulier and Chalikian 2001; Sakurai and Goto 2007). Below pH 2, the volume and compressibility of the protein decrease, providing βlg with a more compact structure (Taulier and Chalikian 2001). It is believed that further structural transition continues at pH values less than 1.0, even though no report exists at the moment. Between pH 2.5 and 8, the overall conformation of the protein is conserved extraordinarily well, despite significant structural changes.

The dimer-to-monomer transition occurring between pH 4.5 and 2.5 is the acid-induced dissociation of the dimeric βlg into monomers. It includes the βlg transition from the monomeric (M) to the acidic form (Q) around pH 3, where the protein is believed to dimerize (Taulier and Chalikian 2001; Sakurai and Goto 2007). This transition triggers a different orientation of the α-helix, which affects only the surface electrostatic properties of βlg. The native dimeric (N) to the acidic (Q) form transition occurs between pH 6.0 and 4.5. This transition is accompanied with a change in the compactness of the protein translated into a slight expansion of the hydrodynamic volume of βlg (Taulier and Chalikian 2001; Sakurai and Goto 2007). It also includes the pH 5 transition (dimer to octamer transition from pH 3.9 to 5), at which βlg undergoes an octamerization without significantly affecting its secondary structure. Above pH 5, the dimerization of βlg is due to the electrostatic interactions between Asp130 and Glu134 of one monomer with corresponding lysyl residues of other monomers. The Tanford (N–R) transition occurs between pH 7 and 8 (Taulier and Chalikian 2001; Sakurai and Goto 2007). This transition involves a conformational change of the E–F loop (residues 85–90), probably due to the cleavage of hydrogen bonds between the F and G strands, as represented in Fig. 7.1 (Sakurai and Goto 2006; Sakurai and Goto 2007). Finally, there is a transition at pH 9.0 or above where βlg undergoes an irreversible base-induced unfolding (Taulier and Chalikian 2001). These structural transitions are important for understanding the functional properties of the protein.

7.3 Functionality of βlg

The physiological role of βlg, albeit not fully understood, seems to be intimately related to its amino acid composition and tertiary structure. The health benefits of βlg and derived peptides go far beyond its undeniable nutritional value and well

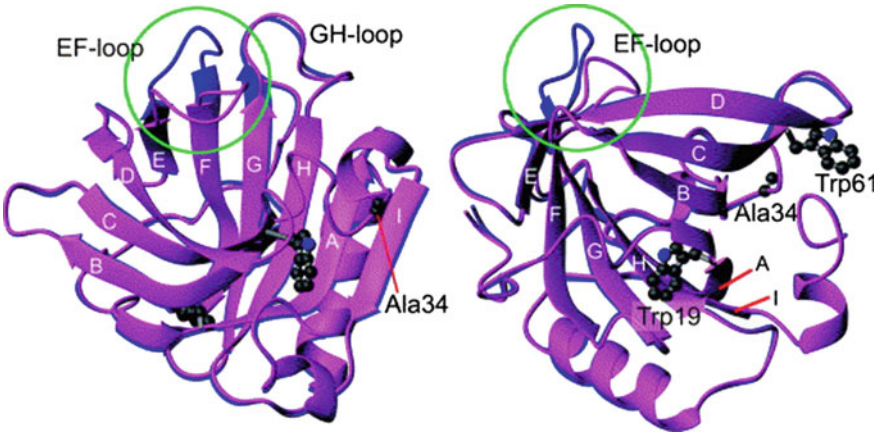

Fig. 7.1 Structures of βlg displaying the E–F loop in the closed (*left*) and open (*right*) conformation in the *green* circle. The conical β-barrel is formed by two sheets consisting of β-strands *A–D* and strands *E–H* [adapted from Sakurai and Goto (2006), Sakurai and Goto (2006)]

justify its use in functional food ingredients and nutraceuticals. Their effects on a number of disease conditions have been reviewed elsewhere (Mehra and O'Kennedy 2009; Morris and FitzGerald 2009). Examples of beneficial activities on human health include their role as hypotensive, anticancer, immunomodulatory, opioid agonist, mineral binding, antimicrobial, gut health-enhancing, hypocholesterolemic, insulinotropic and psychomodulatory agents.

Functional advantages of βlg reside in its capacity for gel formation, foaming and emulsion stabilization, all of which find numerous applications in the food industry (Chen et al. 2006; Tunick 2009; Nicolai et al. 2011). Gelation remains one of the most important techno-functional properties of βlg and is traditionally achieved though thermal treatment. The sequential gelation steps consist of the unfolding of polypeptide chains with concomitant exposure of initially buried hydrophobic amino acid residues and subsequent self-aggregation of protein molecules into a three-dimensional network that entraps water by capillary forces (Chen et al. 2006). Contributing forces are hydrophobic effects, van der Waals interactions, hydrogen bonding and covalent interactions, of which the impacts can be determined via the use of destabilizing agents. As one example, urea can be used to block the formation of the hydrogen bonds, sodium dodecyl sulfate (SDS) to block the formation of the hydrophobic interactions, and 2-mercaptoethanol to block the formation of the disulfide bridge (Remondetto and Subirade 2003). In the absence of salt, βlg forms transparent 'fine-stranded' gels at extreme pH values away from its p*I*, and opaque 'particulate' gels near its p*I*. Salt has been used to induce cold-set gels made by prior heat denaturation of βlg (Remondetto and Subirade 2003; Chen et al. 2006).

The solubility of βlg is greatly enhanced in the presence of salt due its surface charge distribution at neutral pH, thus explaining the harvesting of the protein by dialysis or precipitation upon salting out and further growing of X-ray crystal

structures (Creamer et al. 2011; Sawyer 2013). The structure of βlg justifies its classification as a member of the lipocalin family and calycin subclass, which is naturally involved in the transportation of small hydrophobic bioactives (Sakurai et al. 2009). Reports indicate that βlg binds retinol, triglycerides and long chain fatty acids such as palmitic acid, resulting in enhanced intestinal uptake of these ligands. βlg might also have an important role in carrying implicated ligands in food systems and pharmaceutical preparations, as well as in digestion, absorption and metabolism of some of implicated ligands in neonates (Mehra and O'Kennedy 2009). However, the sequence similarity of βlg with glycodelin, an important protein for fetal development expressed in the endometrium during the first trimester of human pregnancy, suggests further important biological functions (Sakurai and Goto 2006; Sawyer 2013). This is confirmed by the absence of βlg from human and rodent milk and its presence in the whey of ruminants' milk, including cow's milk, thus making its true function still elusive (Bonnaillie and Tomasula 2009; Sawyer 2013). However, there is a general consensus about its role as ligands transporter. Intensive literature exists on the large selection of ligands that bind to βlg (Kontopidis et al. 2004; Creamer et al. 2011; Sawyer 2013). This ligand binding property is mainly ascribed to the presence of the central calyx, which plays the role of a receptacle for hydrophobic and amphiphilic small molecules, consequently forming βlg–ligand complexes (Liang et al. 2008; Liang and Subirade 2010; Diarrassouba et al. 2013).

7.4 Structural Basis for the Formation of the βlg–Ligand Complexes

The folding of the two β-sheets (A–D and E–H) into a central cavity paneled with hydrophobic amino acids resembles a barrel with the EF loop (residues 85–90) acting as the gate (Sakurai et al. 2009). The EF loop folds over the entrance of the calyx to form a closed conformation at pH values lower than 6.5. At pH values above 7, it adopts an open conformation which exposes the interior of the calyx to what? (Qin et al. 1998). This conformational flexibility is attributed to the Tanford transition, which is accompanied by the deprotonation of the carboxyl group of Glu 89, located on the EF loop. Glu 89 that has an anomalous p*Ka* of 7.3 is normally buried and protonated in the 'closed conformation' at acidic pH. At pH values above 7, Glu 89 is exposed to what? and deprotonated, thus triggering the opening of the EF loop. This consequently offers access to the central calyx (Sakurai et al. 2009). The pH-controlled flipping of the EF loop seems to be crucial for the physiological significance of βlg, since in the closed conformation bound ligands might be protected in the acidic stomach in order to be further released within the intestines at higher pH values (Kontopidis et al. 2004; Sakurai and Goto 2007; Sawyer 2013).

A number of methods have been used to study the binding of the ligands to βlg (Collini et al. 2003; Ragona et al. 2003; Riihimäki et al. 2008; Yang et al. 2009; Liang and Subirade 2010; Loch et al. 2011; Diarrassouba et al. 2013). However, spectroscopic methods are among the most user-friendly, rapid, accurate and less cumbersome techniques used to investigate the protein–ligand interaction. Fluorescence spectroscopy is preferred for studying the binding stoichiometric of the βlg–ligand complex, while circular dichroism is interesting for studying the influence of the ligand binding on the secondary and tertiary structures of the protein.

Fluorescence spectroscopy, and in particular fluorescence quenching, is commonly used to characterize ligand binding to proteins. Fluorescence quenching refers to any process that reduces the fluorescence intensity of a sample caused by processes such as the inner-filter effect, energy transfer, ground state complex formation and collisional processes (Zhao et al. 2006; Rouabhia et al. 2007). The inner-filter effect occurs when the fluorescence emission of the fluorophore is affected by the presence of an absorbing substance that absorbs the radiation going towards (excitation) or emanating from (emission) the fluorophore (van de Weert 2010). The resulting fluorescence quenching will thus result from the reduction of the radiation intensity that excites the fluorophore. The inner-effect can be corrected by carefully selecting the concentration of the ligand, so that the absorbance of the ligand at the excitation and emission wavelength is below 0.1 (van de Weert 2010).

Collisional or dynamic quenching results from collisions involving both the fluorophore and the quencher during the lifetime of the excited state, while static quenching refers to ground-state fluorophore–quencher complex formation (Zhao et al. 2006). In the case of collisional quenching, the quencher diffuses to the fluorophore during the lifetime of the excited state and, upon contact, the fluorophore returns to the ground state without emission of a photon (Lakowicz 2006). Important parameters such as the association constant and binding number can be computed using various equations such as the well-known Stern–Volmer equation (in the case of non-fluorescent complex formation) or its modified version derived for a dynamic quenching process, in which there is a constant contribution of fluorescence of the non-quenchable fraction. These equations and variants are well described elsewhere (Zhao et al. 2006; Lakowicz 2006; van de Weert 2010).

Variants of fluorescence spectroscopy include synchronous fluorescence and fluorescence resonance energy transfer (FRET). Synchronous fluorescence is a scan that provides important information about the molecular environment in the vicinity of fluorophores in general, a shift in the position of the emission maximum corresponding to the changes of the polarity around the fluorophore (Guo et al. 2009). The synchronous fluorescence spectra is obtained by recording the fluorescence spectra resulting from the difference between the excitation wavelength and emission wavelength at 15 or 60-nm intervals, corresponding to the changes of the polarity around Tyr and Trp residues, respectively (Guo et al. 2009). Upon ligand binding, a stronger fluorescence quenching for a scan at $\Delta\lambda = 60$ nm (Trp) than for

$\Delta\lambda$ = 15 nm (Tyr) is indicative of the binding of the ligand closer to the Trp residues (Diarrassouba et al. 2013). This information is extremely important since it confirms the formation of a ground-state complex that can be further analyzed in the context of the FRET (Lakowicz 2006; van de Weert 2010). FRET occurs when there is overlap between the fluorescence emission spectrum of a donor fluorophore (in the excited state) with the absorption spectrum of an acceptor ligand (in the ground state). FRET can provide accurate structural information about protein–ligand binding. The transfer rate is affected by any condition that affects the distance between the donor and acceptor biomolecules, owing to FRET the name of 'spectroscopic ruler' (Lakowicz 2006). Thus, the Förster distance, that is the distance for a specific donor–acceptor pair where 50 % of the fluorescence energy of the donor (fluorophore) is transferred to the acceptor (ligand), can be computed to suggest the localization of the ligand on the protein (Lakowicz 2006; van de Weert 2010; Diarrassouba et al. 2013).

7.4.1 Fluorescence Emission of βlg

The fluorescence of *β*lg arises mainly from Trp and Tyr residues. At physiological pH, the dimeric form of *β*lg possesses four Trp residues, two on each monomer (Trp19 and 61). Trp61 is exposed to the solvent, thus explaining the almost complete quenching of its fluorescence. It has also been suggested that the location of Trp61, close to disulfide bond (Cys66–Cys160) and near the guanidine group of Arg124, both considered as strong quenchers, and the possible self-quenching of Trp61 of the other monomer in the *β*lg dimeric form, might all contribute to the reduction of its fluorescence emission (Stănciuc et al. 2012). Trp19 contributes the most (80 %) to the total intrinsic fluorescence, mainly due to its position in the hydrophobic cavity of the native conformation of the protein (Liang et al. 2008). It is thus a highly sensitive probe that is used for monitoring conformational modification in *β*lg. Typically, 280 nm is used as the excitation wavelength with Trp contributing to major fluorescence intensity and a maximum emission peak around 335 nm for *β*lg (Croguennec et al. 2004). While Tyr residues contribute to the fluorescence when exited at 280 nm, only the environment of Trp is studied at the excitation wavelength of 290 nm. The intrinsic fluorescence emission of *β*lg can typically be either quenched or enhanced and shift to a shorter wavelength (blue shift) or a longer wavelength (red shift). Enhancement of the fluorescence emission results from a reduction of intra molecular quenching of Trp residue, for instance when the protein unfolds (Stănciuc et al. 2012). Conversely, quenching occurs when the fluorescence emission of the Trp residue is hindered by the increased intra molecular interactions or upon binding of a ligand. The red shift of the peak indicates that the Trp residue moved from an apolar environment to a more polar region; when surrounded by a more hydrophobic environment, the peaks are blue shifted (Busti et al. 2002; Stănciuc et al. 2012).

7.4.2 Circular Dichroism

Circular dichroism (CD) is extensively used to monitor and understand the structural changes occurring during interactions of proteins with other biological molecules because of its high conformational sensitivity (Woody 2012). The CD signal is a radiation with elliptical polarization that results from the difference of unequal absorption between the left and right components of polarized light (Kelly et al. 2005; Creighton 2010). Proteins exhibit a CD spectrum that is conveniently divided into specific spectral regions, each of which is dominated by different types of chromophores and provides different kinds of information. The secondary structure can be determined by the far UV that ranges from about 170–240 nm and the information is provided by the amide group, which is the dominant chromophores (Diarrassouba et al. 2013). In the near UV range from 250 to 300 nm, protein CD is dominated by aromatic side chains and provides information about tertiary structure (Kelly et al. 2005; Woody 2012; Diarrassouba et al. 2013).

The protein CD spectrum that arises from the far UV is composed of secondary structural elements consisting of α-helix, β-sheets and unordered conformations or random coils. These structural elements in the peptide bonds undergo specific transitions which are well detailed elsewhere (Creighton 2010; Woody 2012). Briefly, it can be indicated that the α-helix characteristic CD spectrum exhibits two negative bands of comparable magnitude at about 222 and 208 nm, plus a stronger positive band near 190 nm. The β-sheet conformation is characterized by two negative bands near 217 and 195 nm and a positive band near 195 nm. Finally, the unordered polypeptides have a weak positive CD band at ∼217 nm and a strong negative band at ∼197 nm (Woody 2012; Nina et al. 2012). The near-UV CD spectra of proteins arise primarily from the packing of side-chain chromophores, including the three aromatic side chains (Phe, Tyr and Trp) and the disulfide group of cysteine (Woody 2012; Nina et al. 2012). The near-UV CD is greatly perturbed by conformational changes or ligands binding in ways that affect the geometry or environment of one or more aromatic side chains (Woody 2012; Diarrassouba et al. 2013). This information is important given that the biological functions of proteins rely on their structural characteristics, thus the smallest change in the structure may result in functional modifications.

7.4.3 βlg–Ligand Complexes

To date, four binding sites have been recognized on βlg, which include the central calyx formed by the β-barrel, the surface hydrophobic pocket in the groove between the α-helix and the β-barrel, the outer surface near tryptophan (Trp)19–arginine (Arg)124, close to the entrance of the β-barrel, and the monomer–monomer interface of the dimer (Liang et al. 2008; Yang et al. 2008; Liang and Subirade 2012). There is a wealth of literature on βlg-based complexes with ligands of

biological importance (Kontopidis et al. 2002; Creamer et al. 2011; Sawyer 2013). βlg can bind ligands of various natures, including metal ions (Divsalar et al. 2012), fatty acids (Kontopidis et al. 2002), vitamins (Yang et al. 2008; Diarrassouba et al. 2013), pharmaceuticals (Agudelo et al. 2012), flavor compounds (Tromelin and Guichard 2006) and polyphenols (Liang et al. 2008; Riihimäki et al. 2008; von Staszewski et al. 2012). This list is far from exhaustive, and research is ongoing for relevant βlg–ligand complexes.

Complexation with βlg is believed to improve the biological properties and the stability to environmental factors of both the ligand and the protein. The pepsin and acid resistance confers to βlg its functional role as carrier of small hydrophobic ligands which are thus protected during transit in the stomach. βlg then releases its cargo charge at higher pH value in the intestine upon proteolitic activity of chymotrypsin, trypsin and minor proteases present in pancreatin (Ragona et al. 2000; Keller 2013). Consequently, a function of βlg might consist in facilitating the digestion of milk fat in neonates (Ragona et al. 2000). It is important to notice that the closed conformation of the EF loop at acidic pH confirms the physiological role of βlg as a transporter of small bioactives, since bound ligands might be protected in the acidic stomach and later be released within the basic small intestine when the EF loop is in the 'open' conformation (Sakurai and Goto 2006; Creamer et al. 2011).

Evidence suggests that binding of ligands to βlg is beneficial for both the protein and bound bioactive molecule. Recently, it was proved that the resistance of βlg to proteolytic activity in the intestines was improved upon binding to vitamin D3 (Diarrassouba et al. 2014). Binding of naturally occurring phosphatidylcholine did not influence the resistance of βlg to gastric pepsinolysis but protected the protein from subsequent degradation under duodenal conditions (Mandalari et al. 2009). It has also been established that complexation with tea polyphenols extracts resulted in an increase in β-sheet and α-helix leading to an alteration of the protein's conformation, which consequently stabilized the structure of βlg (Kanakis et al. 2011). This finding was recently confirmed by the protection of the secondary structure of βlg upon binding to coffee, cocoa and tea polyphenols (Stojadinovic et al. 2013). Substantial research indicates that upon binding to βlg, hydrophobic ligands are better protected against oxidative degradation and the solubility of some of them is enhanced, which might improve the biological properties of the bioactives (Liang et al. 2008; Liang et al. 2011; Diarrassouba et al. 2013). The photostability of folic acid was improved upon binding to the surface of βlg, in the groove between the α-helix and the β-barrel (Liang and Subirade 2010). Riboflavin is a natural occurring photosensitizer that interacts with proteins located in the cell membrane and induces damage to biological systems, including tumor tissues. The binding of riboflavin to βlg leading to formation of the βlg/RF complex exhibited important antiproliferative activity, which was explained by the generation of reactive radical and oxygen species as the result of the interaction between RF and βlg (Diarrassouba et al. 2013). Additionally, it has been clearly established that βlg might have a functional advantage in the transport of vitamin D3, since supplementing milk with vitamin D3 effectively enhances its uptake (Yang et al. 2009).

The βlg–vitamin D3 complex represents an excellent model that confirms the existence of multiple binding sites on the protein. In fact, vitamin D3, α-tocopherol, as well as phosphatidylcholine, each bind to two different sites on βlg: (*i*) the surface hydrophobic pocket in the groove between the α-helix and (*ii*) the β-barrel, and central calyx (Yang et al. 2008; Mandalari et al. 2009; Liang and Subirade 2012). The binding of hydrophobic ligands to the surface site via hydrophobic interactions might provide an additional protection to βlg against proteases due to steric encumbrance (Mandalari et al. 2009). Furthermore, the existence of a secondary surface site might be beneficial to the carrier function of βlg since the disruption of the central calyx upon heat treatment can trigger the release of the bioactive bound inside the cavity (Yang et al. 2008; Liang and Subirade 2012). In fact, βlg undergoes irreversible denaturation with concomitant loss of its tertiary structure above 80 °C, which consequently disallows the binding of the ligands to the central calyx (Sawyer 2013). The binding to the central cavity of βlg can also be hampered by pH-induced transitions and particularly by the Tanford transition. At lower pH values, the EF loop—that is, the gate of the calyx—is in the 'closed conformation', thus preventing binding of the bioactives, which can have access again to the central cavity by raising the pH value (Sakurai et al. 2009; Creamer et al. 2011). Therefore, βlg can be considered as a versatile nano-sized carrier system that can conveniently transport and protect bioactive ligands, whose release can be modulated by controlling environmental factors such as the temperature and pH (Ragona et al. 2003; Liang and Subirade 2012). This property is reinforced by the surface properties of βlg to exhibit charged and neutral amino acid groups upon pH manipulation around its p*I* (5.3). At pH values close to the p*I*, βlg bears zero charge while at pH above and below the p*I*, it is negatively and positively charged, respectively. These physicochemical characteristics confer flexibility in establishing intermolecular interactions (with other βlg molecules) as well as interactions with a wide range of biopolymers. This advantageous property is exploited to fabricate βlg–biopolymer self-assembled structures used in controlled delivery technology.

7.5 The Auto-association of βlg

βlg can self-assemble to form aggregates, depending on protein concentration, ionic forces, pH and temperature of the solution. The mechanisms of βlg self-aggregation have been extensively reviewed (Meredith 2006; Nicolai et al. 2011; Foegeding and Davis 2011; Nicolai and Durand 2013). Thermal gelation of βlg has served in the development of environment-sensitive hydrogels with specific microstructural properties and desired bioactives release profiles (Chen et al. 2006; Gunasekaran et al. 2007). The advantage of thermally induced gels is their capacity to trap bioactive molecules within the gel matrix, stabilize food texture, and to swell in water and hold it in a well-maintained network structure. These characteristics confer protection from hostile environments to bioactives which can thus be released upon environmental triggers such as pH (acidic in the stomach and neutral

in the intestine), temperature and digestive enzymes (Chen et al. 2006). However, heat-sensitive bioactives cannot be encapsulated in thermal gels. This drawback can be bypassed by using cold-set gels, formed by pre-denaturing the proteins using heat treatment and subsequently using salt to induce gelation. Particulate or fine stranded gels sets can then be obtained depending on the ionic force and pH (Remondetto and Subirade 2003). The use of cold-induced gelation of *β*lg for oral delivery of nutraceuticals has been the focus of intense research (Remondetto and Subirade 2003; Remondetto et al. 2004; Sok Line et al. 2005; Chen et al. 2006; Livney 2010). The preheating of *β*lg exposes functional groups to what? which can be used to create interactions with bioactives and unfolded polypeptide chains as well as to confine the size of the delivery matrix into nano- and microparticulate systems.

The size of the biopolymer-based delivery devices can be controlled via two main processes categorized as 'top down' and 'bottom up' approaches (Augustin and Sanguansri 2009). The 'top down' approach consists of breaking up bulk materials into reduced sized matrices, whereas the 'bottom up' approach allows structures to be built from molecules capable of self-assembly (Chen et al. 2006; Augustin and Sanguansri 2009; Verma et al. 2009; Salazar et al. 2012). Food proteins, and particularly *β*lg, have great potential for self-assembly due in part to their polyelectrolytic character, which enables the possibility of conveniently manipulating their surface charge and establishing non-covalent interactions (hydrogen bonding, electrostatic and van der Waals forces). In addition, the presence of thiol groups permits covalent interactions, and lastly, hydrophobic amino acids allow the establishment of hydrophobic interactions upon unfolding of the structure (Singh 2011). The promotion of such interactions between the polypeptides of *β*lg can trigger spontaneous auto-structuration of the protein to form site-specific delivery scaffolds of controlled size.

7.6 *β*lg-Based Delivery Systems: From Molecule to Particles

Generally, nanoparticles refer to functional materials at a length scale of less than 100 nm, although a larger definition includes particles of size inferior to 1 μm (Vo-Dinh 2005; Chen et al. 2006; Sozer and Kokini 2009; Ezhilarasi et al. 2013). Beneficial features of nanoparticles include target and site-specific delivery, ability to penetrate cells and circulating systems, bioactives entrapment and dispersion throughout the dense polymeric network (Gunasekaran et al. 2007). The dense matrix formed by entanglement of polypeptide chains is believed to provide a reinforced resistance to proteolytic attack in simulated gastrointestinal conditions. As such, *β*lg nanoparticles of sub-100-nm size exhibit an improved resistance to digestive proteases at neutral and acidic pH (Gunasekaran et al. 2007). The size of the *β*lg nanoparticles can be reduced to about 60 nm upon preheating of the protein

solution. The use of crosslinking agents such as glutaraldehyde can significantly improve the density of the nanoparticle matrix, which in turn impedes the penetration of the proteolytic enzymes into the platform and subsequently retards the degradation of the protein matrix (Ko and Gunasekaran 2006). Degradation of protein-based delivery platforms by intestinal proteases is a major issue in oral delivery of bioactives, particularly for βlg-based systems. The Tanford transition at intestinal pH provides access for the enzymes trypsin and chymotrypsin to the target amino acid groups, consequently degrading the protein (Sakurai and Goto 2007; Sakurai et al. 2009; Keller 2013). Therefore, the development of βlg-based controlled-release formulations which can efficiently retard the intestinal degradation of are highly advantageous in numerous aspects: (i) increased residence time in the intestines resulting in enhanced adsorption; (ii) improved intimacy of contact with the epithelial membrane and/or at the absorption site; (iii) enhanced bioavailability (Lafitte 2008). The carrier system can be tailored to improve its attachment to the epithelial membrane at the target site, also referred to as mucoadhesion, which is important for enhanced uptake and bioavailability of biomolecules.

Mucoadhesion is an important characteristic that motivated research into βlg-based formulations with improved adhesion to the mucus layer in the intestines (Chen and Subirade 2005; Jones et al. 2009; Zimet and Livney 2009; Livney 2010;). Indeed, the mucus layer represents the first membrane barrier that covers the gastrointestinal (GI) tract, and consequently obstructs direct adhesion to the epithelial cells and hinders the transport of bioactives (Lafitte 2008). The mucus layer is a viscoelastic protective lining of the epithelium, composed mainly of water (∼95 %) and up to 5 % mucins. Mucins are glycoproteins of high molecular weight consisting of a peptide backbone with a significant number of carbohydrate side chains attached to the peptide backbone. Whereas the protein core and formation of the disulfides bridges between the peptide backbones are responsible for the hydrophobic and viscoelastic character of the mucins, respectively, the carboxylic side chains confer a strong negative charge to the mucus layer (Lafitte 2008). Recently, cationic βlg nanoparticles between 75 and 94 nm in size were developed as a bioavailability enhancer for poorly absorbed bioactives (Teng et al. 2013). The cationic βlg nanoparticles were formed by substituting 11 amino acid residues with ethylenediamine, which resulted in positive surface charge and significantly increased surface hydrophobicity. The positively charged βlg nanoparticles improved the mucoadhesion, and were proposed as bioavailability enhancers of nutraceuticals and pharmaceuticals (Teng et al. 2013).

A number of pH-sensitive, biocompatible and biodegradable polysaccharides have been used as mucoadhesive agents, and studies have shown that an increase in charge density enhances the adhesion (George and Abraham 2006; Yu et al. 2009). Interestingly, evidence also indicates that both anionic (e.g. alginate) and cationic (e.g. chitosan) can quite strongly interact with mucins. This can probably be explained by the presence of both charge groups on the backbone and side chains with different pKa, which changes the global charge of the mucin molecules when the pH varies between pH 1 and 7. Thus, while the mucins are fully negatively

charged in the intestines, they carry neutral to weak charge in the stomach, the pKa (2.6) of sialic acid (at the end of the carboxylic groups) being used as a cut-off point (Lafitte 2008). Nano-sized delivery platforms have been developed involving electrostatic complexes and coacervates formation between βlg and polysaccharides. These platforms result from the electrostatic interactions between oppositely charged molecules under particular pH and ionic force conditions (Jones et al. 2009; Chanasattru et al. 2009; Jones et al. 2010a, b; Schmitt and Turgeon 2011). Nanoparticles, with the core constituted of chitosan and shell formed by βlg were developed as nutraceutical carriers (Chen and Subirade 2005). When the native βlg was used to form the shell, the resistance to acid and pepsin degradation property of the protein was preserved.

Heat denatured βlg was used to form electrostatic complexes with pectin, an anionic polysaccharide that is only degraded in the colon by the pectinases and resists proteases and amylase in the upper GI tract (Yu et al. 2009; Jones and McClements 2010). The resulting nanoparticle can be suggested for colon delivery purposes for a wide range of bioactives including pharmaceuticals and nutraceuticals. Vitamin D2, docosahexaenoic acid and major catechin in green tea (–(–)–epigallocatechin-3-gallate) were successfully entrapped in nanoparticles of size varying from 50 to about 100 nm prepared upon promotion of electrostatic interactions between βlg and pectin (Zimet and Livney 2009; Ron et al. 2010; Shpigelman et al. 2012). The authors suggested that these nanovehicles could serve as carriers for hydrophobic nutraceuticals in non-fat foods and clear beverages. The encapsulated bioactives benefitted from significant protection against oxidative degradation, probably due to the mild antioxidant activity of βlg conferred by the free thiol group (Liu et al. 2007; Ron et al. 2010). In addition, the physical entrapment and reduced mobility of light- or oxygen-sensitive bioactives within the protein matrix might provide additional resistance to oxidizing agents such as oxygen or free radicals by restricting their access to the encapsulated bioactive molecule (Ron et al. 2010). The ability of βlg to absorb UV light can also contribute to improving the light stability of the entrapped bioactives that absorb at a proximate wavelength range (Semo et al. 2007). Nanoparticles, particularly those 100 nm or less in size, have the functional advantage of diffusing readily though both the epithelial and lymphatic tissue at target sites where the entrapped bioactive is taken up with high efficiency (Chen et al. 2006; Acosta 2008). However, the release profile of the bioactive can also be effectively modulated by larger submicron particles, which release entrapped bioactive molecules more slowly and over longer periods at the mucosal lining (Chen et al. 2006; Acosta 2008).

7.7 βlg-Based Microparticles for Oral Delivery

Microparticles are spherical shaped scaffolds, less than 1000 μm in size, isolating a variety of sensitive bioactive substances from the surrounding environment by a membrane coating (Nesterenko et al. 2013). Biopolymers and bioactive ingredients

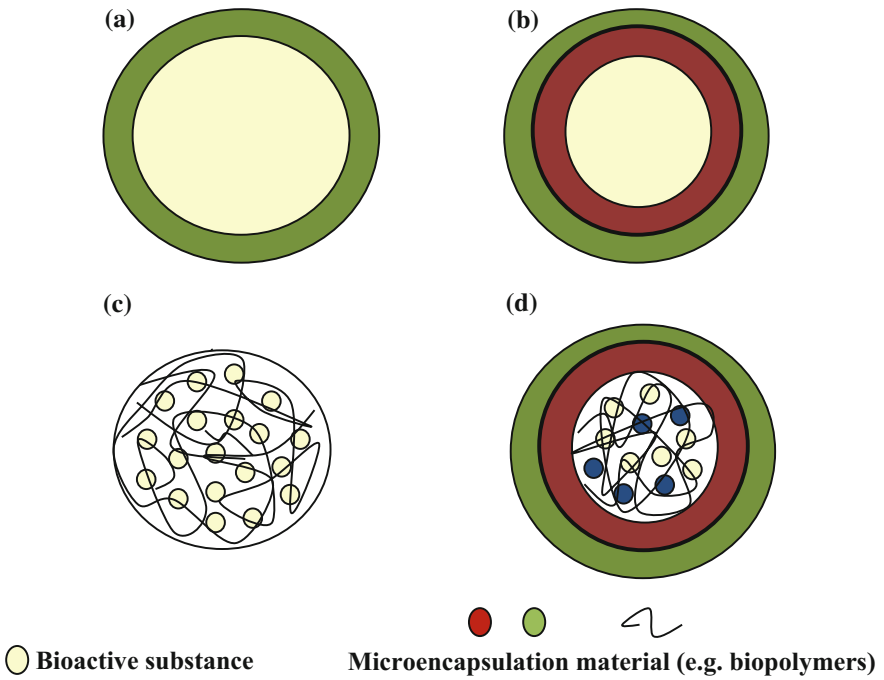

Fig. 7.2 Different structures of microparticles obtained by microencapsulation: **a** microcapsule, **b** multilayer microcapsule, **c** microsphere and **d** multishell and multicore microsphere [adapted from Nesterenko et al. (2013)]

can be combined to form different structures of microparticles including multishell, multilayer microsphere or microcapsule platforms as described in Fig. 7.2.

In the food industry, microparticles have been used for many purposes, including masking unpleasant taste and flavor, converting liquids to solids, and protecting sensitive ingredients from harsh external media including light, moisture, pH and oxidation—this list is far from exhaustive (Chen et al. 2006; Nesterenko et al. 2013). Currently, there is huge number of reports on the use of microparticles as delivery vehicles for nutraceuticals and pharmaceuticals. The use of non-toxic food-grade materials is of particular interest for oral delivery, and a vast volume of research on βlg-based microparticulate systems exists in the literature. Furthermore, βlg belongs to the lipocalin family, which explains its recognition and internalization by the human lipocalin-interacting membrane receptor that is expressed in the intestine (Sawyer 2013). Therefore, its uptake by intestinal cells is advantageous in the formulation of oral delivery platforms that entrap and release bioactives

within the intestines. Microparticles can be formulated to prolong the residence time of bioactives at the target site and improve their bioavailability as a result of enhanced mucoadhesion. Coacervates of βlg with naturally occurring polysaccharides with mucoadhesive properties, such as alginate, pectin, gum arabic and acacia gum, have been used for the encapsulation of probiotics and vitamins (Schmitt et al. 2000; Chen and Subirade 2005; Chen et al. 2006; Turgeon and Laneuville 2009; Schmitt et al. 2009; Hebrard et al. 2010).

Despite important potential applications in delivery technology, the utilization of coacervates is restricted to a narrow window of pH and ionic strength, a range beyond which the microparticles are unstable and thus can lead to premature release of the bioactive cargo charge (Turgeon and Laneuville 2009). Solutions to this issue include formation of Maillard type conjugates consisting of non-enzymatic browning involving the terminal or a side-chain amine group of βlg and the reducing end of a sugar such as dextran (Wooster and Augustin 2006; Caillard et al. 2010). Crosslinking agents including glutaraldehyde or formaldehyde have been used to stabilize coacervates; however, they are toxic and are not permitted for food applications.

Biocompatible alternatives such as rennet, genipin, glyceraldehyde or transglutaminases are all capable of stabilizing coacervates by covalently bridging protein matrices, but with a yield well below that of the toxic ones (Turgeon and Laneuville 2009; Livney 2010). However, it is important to ensure that crosslinking does not impede the bioaccessibility and release of the active molecule entrapped within the carrier matrix, which then forms a barrier against diffusion and inward access of digestive enzymes (Livney 2010). Maillard conjugates, the use of crosslinking agents for improved microparticles stability, and emulsion- and lipid-based particulate systems are beyond the scope of the present review. The formulation of bioactive-loaded carriers with controlled size and release profiles require a full understanding of the behavior of the delivery platform in food systems as well as in the GI tract at the site of absorption. The physicochemical characteristics, including surface properties such as size, surface charge, and interior and surface morphology, are important parameters to consider for optimal bio-interaction, uptake and bioavailability of the bioactive substance.

7.8 Characterization of βlg-Based Nano- and Microparticles

Most βlg-based nano- and microparticulate carriers with a dense matrix result from electrostatic complexes formed between soluble biopolymers. Typically, the self-assembly is promoted upon mixing of clear solutions under controlled pH, temperature and ionic forces conditions. This in turn leads to a modification of the light scattering (turbidity) of the resulting solution, which usually increases rapidly as a consequence of the formation of large aggregates or co-precipitates (Mahler

et al. 2009). The theory behind turbidity measurement is Rayleigh's theory, which indicates that the scattering of light is brought about by particles which are smaller in diameter than the wavelength of the light itself, the upper limit considered to be about one-tenth of the wavelength (Mahler et al. 2009; Moore and Cerasoli 2010). The increase in turbidity is normally associated with an increase in the size of the particles and is commonly measured by UV-vis spectroscopy as optical density in the 340–360-nm range and 550–600 nm (Zimet and Livney 2009; Mahler et al. 2009; Ron et al. 2010). The optical properties of a solution are a function of the particles present to scatter and absorb light (Mahler et al. 2009). However, turbidity data should be interpreted carefully, since various environmental factors including temperature, pH (near the p*I*) and protein concentration can influence the size of βlg–biopolymer self-assembled structures. The size determination is also based on the optical properties of a solution, which are dependent on the ability of the particles dispersed in the solution to scatter and absorb light. Particle size (1–10 μm) and size distribution are frequently determined using laser light scattering, which encompasses static and dynamic light scattering. Detailed information on these optical techniques has been well described in the literature (Murphy and Lee 2006; Mahler et al. 2009; Moore and Cerasoli 2010).

The surface charge of a particle is estimated using the zeta potential that is an indication of the particle stability measured by electrophoretic light scattering (Moore and Cerasoli 2010). Values of zeta potential above +30 mV and below −30 mV are indicative of a stable colloidal system. Instability arises from pH, which affects the surface charge and repulsion/attraction forces between particles, and the ionic strength, which can modify the particle's charge density (Moore and Cerasoli 2010). The interior and surface aspects of particulate systems can be characterized using transmission (TEM) and scanning electron microscopy (SEM), respectively (Chen and Subirade 2005; Chen et al. 2006; Murphy and Lee 2006). Larger-sized particles or visible particles can be observed using optical microscopes equipped with epifluorescence and optical fluorescent filters for improved discrimination between distinct biopolymers, which are stained beforehand (Chen and Subirade 2006; Hebrard et al. 2013). Finally, the encapsulated bioactive can be extracted from the carrier scaffold and its concentration quantitatively determined (encapsulation efficiency) using techniques such as UV-vis spectroscopy at specific wavelengths or high-pressure liquid chromatography (HPLC) (Zimet and Livney 2009; Ron et al. 2010).

The intestinal uptake and bioavailability of a bioactive substance developed for oral administration are commonly evaluated by performing permeability tests using cell cultures or in vivo experiments using animal or human models. The behavior of oral delivery systems in the GI tract and the in vitro bioavailability of bioactives can be monitored using in vitro digestion methods or dissolution tests, following the US Pharmacopeia/Food and Drug Administration (USP/FDA) guidelines (Acosta 2008). Briefly, digestion at 37 °C in an acidic solution (pH 1.2) in the presence of pepsin represents gastric conditions, while digestion at pH 6.8 in the presence of pancreatin simulates intestinal conditions (Chen and Subirade 2006; Acosta 2008; Hebrard et al. 2013). More specifically, the fate of the βlg-based carrier systems in

the stomach and intestines corresponds to the behavior of the protein in different compartments in the GI tract. Native βlg is resistant to acid and pepsin in the stomach, but is degraded at higher pH and by the pancreatin in the intestines (Sakurai et al. 2009; Creamer et al. 2011; Sawyer 2013). Shell/core chitosan/βlg microparticles with the shell formed by native βlg resist under gastric conditions but are degraded in the intestines by enzymes. However, microparticle shells made of denatured βlg have been shown to be rapidly degraded under both gastric and intestinal conditions (Chen and Subirade 2005).

Cell cultures with adenocarcinoma cell lines derived from human colon epithelia (Caco-2 cells) are among the most frequently used means for in vitro permeability tests. The Caco-2 cells have biophysical properties similar to those of the epithelial cells of the intestines and, as such, mimic well the absorption of the bioactives through the intestinal membrane (Acosta 2008). Therefore, Caco-2 cells can be used to evaluate the efficiency of controlled-release formulations (Remondetto et al. 2004; Jiang and Liu 2010; Sun et al. 2012). Better information on the uptake and bioavailability of a bioactive substance can be generated using in vivo experiments with rats, mice or humans. In food systems, the concept of bioavailability is usually erroneously restricted to the biopharmaceutical phase, which includes the release of the bioactive upon physical erosion or chemical degradation of the carrier system, but affords little attention to the biopharmaceutical (distribution, metabolism and elimination) and biological responses of the encapsulated bioactive (Faulks and Southon 2008). Caution should be exercised in interpreting bioavailability data resulting from in vivo experiments. Optimal bioavailability and biological response result necessarily from an accurate understanding of the relationship between the structure of the carrier platform, biophysical characteristics of both the bioactive and encapsulating material, and a good knowledge of physiology of the intended target site.

7.9 Future Trends

The development of protein-based delivery systems is attracting increasing interest, due in part to their GRAS status and the possibility for entrapping bioactives with different physicochemical properties is infinite. In particular, βlg naturally interacts with small ligands as well as food macromolecules to form complexes whose size and surface properties can be controlled. Recent reports indicate that βlg and food proteins including lysozyme, such as egg white proteins, can form electrostatic complexes at pH values between the p*I* of the two biopolymers (Desfougeres et al. 2010). Evidence strongly suggests that the self-assembly of two proteins into delivery scaffolds will constitute a significant research field in the near future, due mostly to the nutritional advantage, generation of bioactive peptides and simplicity of the process, which together offer a variety of applications including bioactive encapsulation and delivery, and protein co-precipitation and isolation. A recent study reported that βlg–lysozyme self-assembled spontaneously to form

microspheres capable of entrapping vitamin D3 at high encapsulation efficiency (Diarrassouba et al. 2014). The microspheres improved the solubility, UV light stability and shelf life in cold storage, and concomitantly enhanced the intestinal uptake and bioavailability of vitamin D3 (Diarrassouba et al. 2014).

Most importantly, protein aggregates and deposition in tissue (known as amyloid fibrils or plaques) have been directly linked with the impairment of cellular functions and the onset of degenerative neurological diseases such as Alzheimer's disease and spongiform encephalopathies (Krebs et al. 2007). The characterization and understanding of food-based protein co-precipitates may providing some insight into the mechanisms leading to the formation of these 'aberrant protein interactions' and resulting severe health conditions (Vabulas and Hartl 2011).

Particulate systems based on the auto-aggregation of βlg can be prepared by conveniently modulating the protein concentration, pH, ionic forces and temperature (Nicolai et al. 2011; Nicolai and Durand 2013). Self-aggregation of denatured βlg was induced upon the addition of calcium to form a particulate system which encapsulated α-tocopherol (Somchue et al. 2009). An alginate coating conferred reinforced protection to the βlg-based particles in the stomach and retarded the release of the vitamin in the intestines. The auto-aggregation of native βlg can be promoted by mild acidification using D-gluconic acid δ-lactone, which is advantageous for heat-sensitive bioactive encapsulation. This βlg-based platform exhibits a dense polypeptide network, in which bioactives will ultimately be bioaccessible by digestive enzymes, offering significant protective barriers against damaging environmental factors including light and oxidative species. Recently, a βlg-based platform was prepared by inducing the auto-aggregation of protein near its p*I* (Diarrassouba et al. 2014), and vitamin D3 was successfully entrapped within the matrix of platform. As a result, the solubility, UV light stability and long term storage in the cold were significantly improved.

Animal proteins have attracted much attention, mainly due to their excellent techno-functional properties, biocompatibility, biodegradability and GRAS (generally recognized as safe) status (Livney 2010; Elzoghby et al. 2012). Animal proteins collagen and gelatin, a hydrolysis product of collagen, have been widely used for oral drug delivery (Elzoghby et al. 2012). However, they have weak mechanical properties, making the use of harmful crosslinking agents such as glutaraldehyde necessary to enhance the stability of collagen and gelatin–based oral delivery systems. Although other, less toxic crosslinking agents exist, they are not readily available (Liang et al. 2003; Sehgal and Srinivasan 2009). Other animal proteins, such as silk proteins produced by larva silkworms or small insects such as spiders, and elastin or recombinant elastin, have all been used to prepare micro- or nanoparticulate oral delivery systems, which are well summarized by Elzoghby et al. (2012). However, these proteins are expensive, and producing industrial amounts may not be cost-effective. Furthermore, the implication of animal proteins in the spreading of neurodegenerative diseases such as bovine spongiform encephalitis (mad cow disease) and potential risks for allergic reactions have prompted a search for alternatives from natural sources such as vegetable proteins (Elzoghby et al. 2012).

Plant proteins are biodegradable and from abundant and renewable sources, with interesting techno-functional and physicochemical properties that are currently under intensive scrutiny for nano- and microencapsulation of bioactives (Elzoghby et al. 2012; Nesterenko et al. 2013). Protein from soy beans, peas, corn, wheat, barley, rice, oats and sunflowers have all been investigated for the development of encapsulation matrices (Chen and Subirade 2009; Chen et al. 2010; Wang et al. 2011a, b; Elzoghby et al. 2012; Nesterenko et al. 2013). For instance, zein from corn kernels, gliadin from wheat gluten and soy protein have been used for microsphere and nanoparticle formulation (Chen and Subirade 2009; Chen et al. 2010; Luo et al. 2012). However, they are highly hydrophobic, which restricts their carrier ability and they are soluble mostly in organic solvents, which may not be compatible with fragile bioactive molecules, in addition to being sensitive to the gastric environment (Elzoghby et al. 2012). Despite their reduced production cost compared to animal proteins, plant proteins are highly hydrophobic which require the use of potentially toxic organic solvents. However, this hydrophobic character is useful for avoiding the use of crosslinking agents, which is beneficial for oral delivery of bioactives (Elzoghby et al. 2012).

7.10 Sources of Further Information

- Milk proteins: Handbook of hydrocolloid 2009 (O'Regan et al. 2009; Boland 2011).
- Whey processing, functionality and health benefits. John Wiley & Sons (Onwulata and Huth 2008).
- Whey protein fractionation (Bonnaillie and Tomasula 2009).
- Detailed study on βlg structure and folding (Sakurai et al. 2009).
- Spectroscopic methods: The principle of fluorescence spectroscopy (Lakowicz 2006) and Circular Dichroism (Creighton 2010; Nina et al. 2012).
- Delivery and Controlled Release of Bioactives in Foods and Nutraceuticals (Garti 2008).

References

Acosta E (2008) Testing the effectiveness of nutrient delivery systems. In: Garti N (ed) Delivery and controlled release of bioactives in foods and nutraceuticals. Woodhead Publishing, New York, pp 53–106

Agudelo D, Beauregard M, Bérubé G, Tajmir-Riahi H-A (2012) Antibiotic doxorubicin and its derivative bind milk β-lactoglobulin. J Photoch Photobio B 117:185–192

Augustin MA, Sanguansri P (2009) Chapter 5—nanostructured materials in the food industry. In: Steve LT (ed) Adv. Academic Press, Food Nutr Res, pp 183–213

Beaulieu L, Savoie L, Paquin P, Subirade M (2002) Elaboration and characterization of whey protein beads by an emulsification/cold gelation process: application for the protection of retinol. Biomacromolecules 3:239–248

Boland M (2011) Whey proteins. In: Phillips GO, Williams PA (ed) Handbook of food proteins. Woodhead Publishing, USA, pp 32–55

Bonnaillie LM, Tomasula PM (2009) Whey protein fractionation. In: Onwulata C, Huth P (eds) Whey Processing. Wiley, Functionality and health benefits, pp 15–38

Botelho MM, Valente-Mesquita VL, Oliveira KMG, Polikarpov I, Ferreira ST (2000) Pressure denaturation of β-lactoglobulin. Eur J Biochem 267:2235–2241

Busti P, Scarpeci S, Gatti CA, Delorenzi NJ (2002) Use of fluorescence methods to monitor unfolding transitions in β-lactoglobulin. Food Res Int 35:871–877

Caillard R, Mateescu MA, Subirade M (2010) Maillard-type cross-linked soy protein hydrogels as devices for the release of ionic compounds an in vitro study. Food Res Int 43:2349–2355

Chanasattru W, Jones OG, Decker EA, McClements DJ (2009) Impact of cosolvents on formation and properties of biopolymer nanoparticles formed by heat treatment of β-lactoglobuline—Pectin complexes. Food Hydrocoll 23:2450–2457

Chen L, Subirade M (2005) Chitosan/β-lactoglobulin core–shell nanoparticles as nutraceutical carriers. Biomaterials 26:6041–6053

Chen L, Subirade M (2006) Alginate-whey protein granular microspheres as oral delivery vehicles for bioactive compounds. Biomaterials 27:4646–4654

Chen L, Subirade M (2009) Elaboration and characterization of soy/zein protein microspheres for controlled nutraceutical delivery. Biomacromolecules 10:3327–3334

Chen L, Remondetto GE, Subirade M (2006) Food protein-based materials as nutraceutical delivery systems. Trends Food Sci Tech 17:272–283

Chen L, Hebrard G, Beyssac E, Denis S, Subirade M (2010) In vitro study of the release properties of soy-zein protein microspheres with a dynamic artificial digestive system. J Agric Food Chem 58:9861–9867

Collini M, D'Alfonso L, Molinari H, Ragona L, Catalano M, Baldini G (2003) Competitive binding of fatty acids and the fluorescent probe 1-8-anilinonaphthalene sulfonate to bovine β-lactoglobulin. Protein Sci 12:1596–1603

Creamer LK, Loveday SM, Sawyer L (2011) Milk proteins| β-lactoglobulin. In: John WF (ed) Editor-in-chief: encyclopedia of dairy sciences (Second Edition). Academic Press, San Diego, pp 787–794

Creighton TE (2010) Physical and chemical basis of molecular biology. In: Creighton TE (ed). Helvetian Press, New York, pp 681

Croguennec T, Molle D, Mehra R, Bouhallab S (2004) Spectroscopic characterization of heat-induced nonnative beta-lactoglobulin monomers. Protein Sci 13:1340–1346

Desfougeres Y, Croguennec T, Lechevalier V, Bouhallab S, Nau F (2010) Charge and size drive spontaneous self-assembly of oppositely charged globular proteins into microspheres. J Phys Chem B 114:4138–4144

Diarrassouba F, Liang L, Remondetto GE, Subirade M (2013a) Nanocomplex formation between riboflavin and β-lactoglobulin: Spectroscopic investigation and biological characterization. Food Res Int 52:557–567

Diarrassouba F, Remondetto GE, Liang L, Garrait G, Beyssac E, Subirade M (2013b) Effects of gastrointestinal pH conditions on the stability of the β-lactoglobulin/vitamin D3 complex and on the solubility of vitamin D3. Food Res Int 52:515–521

Diarrassouba F, Garrait G, Remondetto GE, Alvarez P, Beyssac E, Subirade M (2014a) Increased stability and protease resistance of the β-lactoglobulin/vitamin D3 complex. Food Chem 145:646–652

Diarrassouba F, Remondetto G, Garrait G, Alvarez P, Beyssac E, Subirade M (2015) Self-assembly of β-lactoglobulin and egg white lysozyme as a potential carrier for nutraceuticals. Food Chem 173:203–209

Diarrassouba F, Garrait G, Remondetto GE, Alvarez P, Beyssac E, Subirade M (2014) Increased water solubility, stability and bioavailability of vitamin D3 upon sequestration in β-lactoglobulin—based coagulum, (Unpublished)

Divsalar A, Barzegar L, Behbehani GR (2012) Thermal study of a newly synthesized cu(ii) complex binding to bovine β-lactoglobulin. J Chem 2013

Elzoghby AO, Samy WM, Elgindy NA (2012) Protein-based nanocarriers as promising drug and gene delivery systems. J Control Release 161:38–49

Ezhilarasi PN, Karthik P, Chhanwal N, Anandharamakrishnan C (2013) Nanoencapsulation techniques for food bioactive components: a review. Food Bioprocess Technol 6:628–647

Faulks RM, Southon S (2008) Assessing the bioavailability of nutraceuticals. In: Garti N (ed) Delivery and controlled release of bioactives in foods and nutraceuticals. Woodhead Publishing, New York, pp 3–25

Foegeding EA, Davis JP (2011) Food protein functionality: a comprehensive approach. Food Hydrocoll 25:1853–1864

Garti N (2008) Delivery and controlled release of bioactives in foods and nutraceuticals. Woodhead Publishing, New York

George M, Abraham TE (2006) Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan—a review. J Control Release 114:1–14

Gunasekaran S, Ko S, Xiao L (2007) Use of whey proteins for encapsulation and controlled delivery applications. J Food Eng 83:31–40

Guo XJ, Sun XD, Xu SK (2009) Spectroscopic investigation of the interaction between riboflavin and bovine serum albumin. J Mol Struct 931:55–59

H.A. Schiffter, 5.46 - The Delivery of Drugs – Peptides and Proteins, in: M.-Y. Editor-in-Chief: Murray (Ed.) Comprehensive Biotechnology (Second Edition), Academic Press, Burlington, 2011, pp. 587–604

Health Canada (2013) A new approach to natural health products. Available at: http://www.hc-sc.gc.ca/dhp-mps/prodnatur/nhp-new-nouvelle-psn-eng.php#fnb1-ref. Accessed on 05 Aug 2013

Hebrard G, Blanquet S, Beyssac E, Remondetto G, Subirade M, Alric M (2006) Use of whey protein beads as a new carrier system for recombinant yeasts in human digestive tract. J Biotechnol 127:151–160

Hebrard G, Hoffart V, Cardot JM, Subirade M, Alric M, Beyssac E (2009) Investigation of coated whey protein/alginate beads as sustained release dosage form in simulated gastrointestinal environment. Drug Dev Ind Pharm 35:1103–1112

Hebrard G, Hoffart V, Beyssac E, Cardot JM, Alric M, Subirade M (2010) Coated whey protein/alginate microparticles as oral controlled delivery systems for probiotic yeast. J Microencapsul 27:292–302

Hebrard G, Hoffart V, Cardot JM, Subirade M, Beyssac E (2013) Development and characterization of coated-microparticles based on whey protein/alginate using the Encapsulator device. Drug Dev Ind Pharm 128–137

IFST, Nanotechnology (2006). Information statement, In: P.A.a.T.L. Committees (ed) Institute of food science & technology, London, UK

Jiang HR, Liu N (2010) Self-assembled β-lactoglobulin–conjugated linoleic acid complex for colon cancer-targeted substance. J Dairy Sci 93:3931–3939

Jones OG, McClements DJ (2010) Biopolymer nanoparticles from heat-treated electrostatic protein–polysaccharide complexes: factors affecting particle characteristics. J Food Sci 75: N36–N43

Jones OG, Decker EA, McClements DJ (2009) Formation of biopolymer particles by thermal treatment of beta-lactoglobulin-pectin complexes. Food Hydrocoll 23:1312–1321

Jones OG, Decker EA, McClements DJ (2010a) Comparison of protein–polysaccharide nanoparticle fabrication methods: impact of biopolymer complexation before or after particle formation. J Colloid Interface Sci 344:21–29

Jones OG, Lesmes U, Dubin P, McClements DJ (2010b) Effect of polysaccharide charge on formation and properties of biopolymer nanoparticles created by heat treatment of beta-lactoglobulin-pectin complexes. Food Hydrocoll 24:374–383

Kanakis CD, Hasni I, Bourassa P, Tarantilis PA, Polissiou MG, Tajmir-Riahi H-A (2011) Milk β-lactoglobulin complexes with tea polyphenols. Food Chem 127:1046–1055
Keller J (2013) Gastrointestinal digestion and absorption. In: William JL, Lane MD (ed) Editors-in-chief: encyclopedia of biological chemistry. Academic Press, Waltham, pp 354–359
Kelly SM, Jess TJ, Price NC (2005) How to study proteins by circular dichroism. BBA Proteins Proteom 1751:119–139
Kim KK, Pack DW (2006) Microspheres for drug delivery bioMEMS and biomedical nanotechnology. In: Ferrari M, Lee AP, Lee LJ (eds), Springer US, pp 19–50
Ko S, Gunasekaran S (2006) Preparation of sub-100-nm β-lactoglobulin (BLG) nanoparticles. J Microencapsul 23:887–898
Kontopidis G, Holt C, Sawyer L (2002) The ligand-binding site of bovine β-lactoglobulin: evidence for a function? J Mol Biol 318:1043–1055
Kontopidis G, Holt C, Sawyer L (2004) Invited review: β-lactoglobulin: binding properties, structure, and function. J Dairy Sci 87:785–796
Krebs MRH, Devlin GL, Donald AM (2007) Protein particulates: another generic form of protein aggregation? Biophys J 92:1336–1342
Lafitte G (2008) Structure of the gastrointestinal mucus layer and implications for controlled release and delivery of functional food ingredients. In: Garti N (ed) Delivery and controlled release of bioactives in foods and nutraceuticals. Woodhead Publishing, New York, pp 26–52
Lakowicz JR (2006a) Principles of fluorescence spectroscopy. In: Lakowicz JR (ed) Springer. USA, US, Baltimore, MD
Lakowicz JR (2006) Energy transfer, In: Springer (ed) Principles of fluorescence spectroscopy, New York, USA, pp 443–475
Liang L, Subirade M (2010) β-lactoglobulin/folic acid complexes: formation, characterization, and biological implication. J Phys Chem B 114:6707–6712
Liang L, Subirade M (2012) Study of the acid and thermal stability of β-lactoglobulin–ligand complexes using fluorescence quenching. Food Chem 132:2023–2029
Liang H-C, Chang W-H, Lin K-J, Sung H-W (2003) Genipin-crosslinked gelatin microspheres as a drug carrier for intramuscular administration: In vitro and in vivo studies. J Biomed Mater Res A 65A:271–282
Liang L, Tajmir-Riahi HA, Subirade M (2008) Interaction of β-lactoglobulin with resveratrol and its biological implications. Biomacromolecules 9:50–56
Liang L, Tremblay-Hebert V, Subirade M (2011) Characterisation of the beta-lactoglobulin/alpha-tocopherol complex and its impact on alpha-tocopherol stability. Food Chem 126:821–826
Liu HC, Chen WL, Mao SJT (2007) Antioxidant nature of bovine milk β-Lactoglobulin. J Dairy Sci 90:547–555
Livney YD (2010) Milk proteins as vehicles for bioactives. Curr Opin Colloid In 15:73–83
Loch J, Polit A, Górecki A, Bonarek P, Kurpiewska K, Dziedzicka-Wasylewska M, Lewiński K (2011) Two modes of fatty acid binding to bovine β-lactoglobulin—crystallographic and spectroscopic studies. J Mol Recognit 24:341–349
Luo Y, Teng Z, Wang Q (2012) Development of zein nanoparticles coated with carboxymethyl chitosan for encapsulation and controlled release of vitamin D3. J Agric Food Chem 60:836–843
Mahler H-C, Friess W, Grauschopf U, Kiese S (2009) Protein aggregation: pathways, induction factors and analysis. J Pharm Sci 98:2909–2934
Mandalari G, Mackie AM, Rigby NM, Wickham MSJ, Mills ENC (2009) Physiological phosphatidylcholine protects bovine β-lactoglobulin from simulated gastrointestinal proteolysis. Mol Nutr Food Res 53:S131–S139
Mehra R, O'Kennedy BT (2009) Separation of β-Lactoglobulin from whey: its physico-chemical properties and potential uses. In: Huth CIOaPJ (ed) Whey processing, functionality and health benefits. Wiley-Blackwell, Oxford, UK, pp 39–62
Meredith SC (2006) Protein denaturation and aggregation. Ann N Y Acad Sci 1066:181–221

Moore J, Cerasoli E (2010) Particle light scattering methods and applications. In: John L (ed) Editor-in-chief: encyclopedia of spectroscopy and spectrometry (Second Edition). Academic Press, Oxford, pp 2077–2088

Morris PE, FitzGerald RJ (2009) Whey Proteins and Peptides in Human Health. In: Onwulata C, Huth P (eds) Whey processing. Wiley, Functionality and Health Benefits, pp 285–384

Murphy RM, Lee CC (2006) Laser light scattering as an indispensable tool for probing protein aggregation. In: Misbehaving proteins. Springer, New York, pp 147–165

Nesterenko A, Alric I, Silvestre F, Durrieu V (2013) Vegetable proteins in microencapsulation: a review of recent interventions and their effectiveness. Ind Crops Prod 42:469–479

Nicolai T, Durand D (2013) Controlled food protein aggregation for new functionality. Curr Opin Colloid Interface Sci 18:249–256

Nicolai T, Britten M, Schmitt C (2011) β-Lactoglobulin and WPI aggregates: formation, structure and applications. Food Hydrocoll 25:1945–1962

Nina B, Polavarapu PL, Nakanishi K, Woody RW (2012) Comprehensive chiroptical spectroscopy: applications in stereochemical analysis of synthetic compounds, natural products, and biomolecules. In: Nina Berova PLP, Nakanishi K, Woody RW (ed) Comprehensive chiroptical spectroscopy. Wiley, New York

Núñez Sellés AJ (2011) Natural health products (NHPs), In: Nriagu JO (ed) Encyclopedia of environmental health, Elsevier, Burlington, pp 33–43

Oliveira KMG, Valente-Mesquita VL, Botelho MM, Sawyer L, Ferreira ST, Polikarpov I (2001) Crystal structures of bovine β-lactoglobulin in the orthorhombic space group C2221. Eur J Biochem 268:477–484

Onwulata CI, Huth PJ (2008) Whey processing, functionality and health benefits. Wiley, New York

O'Regan J, Ennis MP, Mulvihill DM (2009) Milk proteins. In: Phillips PAWGO (ed) Handbook of hydrocolloids. Woodhead Publishing Limited, Cambridge, UK, pp 298–358

Qin BY, Bewley MC, Creamer LK, Baker HM, Baker EN, Jameson GB (1998) Structural basis of the tanford transition of bovine β-lactoglobulin. Biochemistry 37:14014–14023

Ragona L, Zetta L, Fogolari F, Molinari H, Pérez DM, Puyol P, Kruif KD, Löhr F, Rüterjans H (2000) Bovine β-lactoglobulin: Interaction studies with palmitic acid. Protein Sci 9:1347–1356

Ragona L, Fogolari F, Catalano M, Ugolini R, Zetta L, Molinari H (2003) EF loop conformational change triggers ligand binding in β-lactoglobulins. J Biol Chem 278:38840–38846

Ranade V, Cannon J (2011) B., Oral Drug Delivery. In: Press C (ed) Drug delivery systems. CRC Press Talyors & Francis Group, Boca Raton, FL, USA, pp 169–238

Remondetto GE, Subirade M (2003) Molecular mechanisms of Fe^{2+} -induced beta-lactoglobulin cold gelation. Biopolymers 69:461–469

Remondetto GE, Beyssac E, Subirade M (2004) Iron availability from whey protein hydrogels: an in vitro study. J Agric Food Chem 52:8137–8143

Riihimäki LH, Vainio MJ, Heikura JMS, Valkonen KH, Virtanen VT, Vuorela PM (2008) Binding of phenolic compounds and their derivatives to bovine and reindeer β-lactoglobulin. J Agric Food Chem 56:7721–7729

Ron N, Zimet P, Bargarum J, Livney YD (2010) Beta-lactoglobulin–polysaccharide complexes as nanovehicles for hydrophobic nutraceuticals in non-fat foods and clear beverages. Int Dairy J 20:686–693

Rouabhia M, Gilbert V, Wang H, Subirade M (2007) In vivo evaluation of whey protein-based biofilms as scaffolds for cutaneous cell cultures and biomedical applications. Biomed Mater 2: S38–S44

Sakurai K, Goto Y (2006) Dynamics and mechanism of the tanford transition of bovine β-lactoglobulin studied using heteronuclear NMR spectroscopy. J Mol Biol 356:483–496

Sakurai K, Goto Y (2007) Principal component analysis of the pH-dependent conformational transitions of bovine β-lactoglobulin monitored by heteronuclear NMR. Proc Natl Acad Sci U S A 104:15346–15351

Sakurai K, Konuma T, Yagi M, Goto Y (2009) Structural dynamics and folding of beta-lactoglobulin probed by heteronuclear NMR. BBA General Subj 1790:527–537

Salazar J, Ghanem A, Müller RH, Möschwitzer JP (2012) Nanocrystals: comparison of the size reduction effectiveness of a novel combinative method with conventional top–down approaches. Eur J Pharm Biopharm 81:82–90

Santus G, Baker RW (2003) Pharmaceuticals, controlled release of. In: Robert AM (ed) Editor-in-chief: encyclopedia of physical science and technology (Third Edition), Academic Press, New York, pp 791–803

Sawyer L (2013) β-Lactoglobulin. In: Paul PFF, McSweeney LH (ed) Advanced dairy chemistry, Springer US, pp 211–259

Schmitt C, Turgeon SL (2011) Protein/polysaccharide complexes and coacervates in food systems. Adv Colloid Interface Sci 167:63–70

Schmitt C, Sanchez C, Despond S, Renard D, Thomas F, Hardy J (2000) Effect of protein aggregates on the complex coacervation between β-lactoglobulin and acacia gum at pH 4.2. Food Hydrocoll 14:403–413

Schmitt C, Aberkane, Sanchez C (2009) Protein—polysaccharide complexes and coacervates. In: Handbook of hydrocolloids, pp 420–476

Sehgal PK, Srinivasan A (2009) Collagen-coated microparticles in drug delivery. Expert Opin Drug Deliv 6:687–695

Semo E, Kesselman E, Danino D, Livney YD (2007) Casein micelle as a natural nano-capsular vehicle for nutraceuticals. Food Hydrocoll 21:936–942

Shpigelman A, Cohen Y, Livney YD (2012) Thermally-induced β-lactoglobulin–EGCG nanovehicles: loading, stability, sensory and digestive-release study. Food Hydrocoll 29:57–67

Singh H (2011) Milk protein products| functional properties of milk proteins. In: John WF (ed) Editor-in-chief: encyclopedia of dairy sciences (Second Edition), Academic Press, San Diego, pp 887–893

Sok Line VL, Remondetto GE, Subirade M (2005) Cold gelation of β-lactoglobulin oil-in-water emulsions, Food Hydrocoll, 19:269–278

Somchue W, Sermsri W, Shiowatana J, Siripinyanond A (2009) Encapsulation of α-tocopherol in protein-based delivery particles. Food Res Int 42:909–914

Sozer N, Kokini JL (2009) Nanotechnology and its applications in the food sector. Trends Biotechnol 27:82–89

Stănciuc N, Aprodu I, Râpeanu G, Bahrim G (2012) Fluorescence spectroscopy and molecular modeling investigations on the thermally induced structural changes of bovine β-lactoglobulin. Innov Food Sci Emerg Technol 15:50–56

Stojadinovic M, Radosavljevic J, Ognjenovic J, Vesic J, Prodic I, Stanic-Vucinic D, Cirkovic Velickovic T (2013) Binding affinity between dietary polyphenols and β-lactoglobulin negatively correlates with the protein susceptibility to digestion and total antioxidant activity of complexes formed. Food Chem 136:1263–1271

Sun FS, Ju CX, Chen JH, Liu S, Liu N, Wang KK, Liu CG (2012) Nanoparticles based on hydrophobic alginate derivative as nutraceutical delivery vehicle: vitamin D-3 loading. Artif Cells Blood Substit Immobil Biotechnol 40:113–119

Taulier N, Chalikian TV (2001) Characterization of pH-induced transitions of β-lactoglobulin: ultrasonic, densimetric, and spectroscopic studies. J Mol Biol 314:873–889

Teng Z, Li Y, Luo Y, Zhang B, Wang Q (2013) Cationic beta-lactoglobulin nanoparticles as a bioavailability enhancer: protein characterization and particle formation. Biomacromolecules 3:3

Tromelin A, Guichard E (2006) Interaction between flavour compounds and β-lactoglobulin: approach by NMR and 2D/3D-QSAR studies of ligands. Flavour Fragr J 21:13–24

Tunick MH (2009) Whey protein production and utilization: a brief history. In: Huth CIOaPJ (ed) Whey processing, functionality and health benefits. Wiley-Blackwell, Oxford, UK, pp. 1–13

Turgeon SL, Laneuville SI (2009) Protein + polysaccharide coacervates and complexes: from scientific background to their application as functional ingredients in food products In: Stefan K, Ian TN, Johan ITN B Kasapis S, Johan BU (eds) UbbinkA2—Modern biopolymer science. Academic Press, San Diego, pp 327–363

Uhrínová S, Smith MH, Jameson GB, Uhrín D, Sawyer L, Barlow PN (2000) Structural changes accompanying pH-induced dissociation of the β-lactoglobulin dimer. Biochemistry 39:3565–3574

Vabulas RM, Hartl FU (2011) Aberrant protein interactions in amyloid disease. Cell Cycle 10:1512–1513

van de Weert M (2010) Fluorescence quenching to study protein-ligand binding: common errors. J Fluoresc 20:625–629

Verma S, Gokhale R, Burgess DJ (2009) A comparative study of top-down and bottom-up approaches for the preparation of micro/nanosuspensions. Int J Pharm 380:216–222

Vo-Dinh T (2005) Protein nanotechnology. In: Vo-Dinh T (ed) Protein nanotechnology. Humana Press, New York, pp 1–13

von Staszewski M, Jara FL, Ruiz ALTG, Jagus RJ, Carvalho JE, Pilosof AMR (2012) Nanocomplex formation between β-lactoglobulin or caseinomacropeptide and green tea polyphenols: impact on protein gelation and polyphenols antiproliferative activity. J Funct Foods 4:800–809

Wang R, Tian ZG, Chen L (2011a) A novel process for microencapsulation of fish oil with barley protein. Food Res Int, 44:2735–2741

Wang R, Tian Z, Chen L (2011b) Nano-encapsulations liberated from barley protein microparticles for oral delivery of bioactive compounds. Int J Pharm, 406:153–162

Weiss CH, Takhistov P, McClements DJ (2006) Functional materials in food nanotechnology. J Food Sci 71:R107–R116

Woody RW (2012) Electronic circular dichroism of proteins. In: Nina Berova PLP, Nakanishi K, Woody RW (ed) Comprehensive chiroptical spectroscopy. Wiley, New York, pp 473–497

Wooster TJ, Augustin MA (2006) β-lactoglobulin-dextran Maillard conjugates: their effect on interfacial thickness and emulsion stability. J Colloid Interface Sci 303:564–572

Yang M-C, Guan H-H, Liu M-Y, Lin Y-H, Yang J-M, Chen W-L, Chen C-J, Mao SJT (2008a) Crystal structure of a secondary vitamin D3 binding site of milk β-lactoglobulin. Proteins 71:1197–1210

Yang MC, Guan HH, Yang JM, Ko CN, Liu MY, Lin YH, Huang YC, Chen CJ, Mao SJT (2008b) Rational design for crystallization of β-lactoglobulin and vitamin D3 complex: Revealing a secondary binding site Cryst. Growth Des. 8:4268–4276

Yang MC, Chen NC, Chen CJ, Wu CY, Mao SJ (2009) Evidence for beta-lactoglobulin involvement in vitamin D transport in vivo–role of the gamma-turn (Leu-Pro-Met) of beta-lactoglobulin in vitamin D binding. FEBS J 276:2251–2265

Yu C-Y, Yin B-C, Zhang WN, Cheng S-X, Zhang X-Z, Zhuo R-X (2009) Composite microparticle drug delivery systems based on chitosan, alginate and pectin with improved pH-sensitive drug release property. Colloids Surf B Biointerfaces 68:245–249

Zhao H, Ge M, Zhang Z, Wang W, Wu G (2006) Spectroscopic studies on the interaction between riboflavin and albumins. Spectrochim Acta A 65:811–817

Zimet P, Livney YD (2009) Beta-lactoglobulin and its nanocomplexes with pectin as vehicles for ω-3 polyunsaturated fatty acids. Food Hydrocoll 23:1120–1126

Chapter 8
Crystallization

Nicolas Verhoeven, Tze Loon Neoh, Takeshi Furuta and Hidefumi Yoshii

Abstract Ethanol-mediated crystal transformation is a promising method for obtaining stable anhydrous crystals with a porous structure. The DSC (Differential scanning calorimetry)-coupled ethanol method was used to obtain anhydrous trehalose crystals with a specific size and shape by controlling the water content of the ethanol. DSC analysis of the crystal transformation reaction of trehalose or maltose under isothermal and non-isothermal conditions allowed an overall evaluation of the activation energy of crystal transformation, and presumably revealed two different transformation reactions in the glassy state before the click point temperatures and the rubbery state after the click point temperatures. Anhydrous sugar crystals with an increased specific surface area were produced via ethanol-mediated crystal transformation under appropriate conditions. These porous crystals with high surface area could be applied to encapsulate flavor or functional foods. Thin needle-shaped sugar crystals can also be used to form a creamy, non-oil gel in over supersaturated solution conditions.

Keywords Trehalose · Sugar · Ethanol · Dehydration anhydrous · Hydrous · Crystal transformation

8.1 Introduction

Sugars are among the most important major components in food. Sugars come naturally in their hydrous forms. This study includes (1) the concept and molecular arrangement of hydrous crystal sugar or amorphous solid to the formation of anhydrous crystal sugar, and (2) the concept of physical property changes and

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molecular mobility around the glass transition, as well as mathematical descriptions of crystal transformation and influences on food stability.

Diffusion of water in foods is important in product development, processing, and storage design. The rate of crystal structure changes often depends on the physical state and, therefore, on temperature and water content.

Crystal forms are very useful in the food industry for storing sugar or salt food additives. Hartel and Shastry (1991) reviewed sugar crystallization in food products, with an emphasis on the relationship between these crystallization phenomena and the solution structure for comparison purposes. The effects of fat crystals in food emulsion formation and stability are reviewed by Rousseau (2000). During processing and/or storage, intraglobular fat (e.g. in cream) may solidify, forming crystals that can protrude through the interface, leading to droplet coalescence (Boode et al. 1991). There are several papers on fat crystal and water-in-oil emulsion stability (Supratim and Rousseau 2011), but little is known about the crystal transformation of sugars. Crystalline sugars typically exist in anhydrate and hydrated forms, such as lactose and lactose monohydrate, glucose and glucose monohydrate, maltose and maltose monohydrate, trehalose and trehalose dihydrates, α-, β-, and γ-cyclodextrins (CDs), and hexa-, dodeca-, and higher hydrates (Nakai et al. 1986). It is well known that hydrous and anhydrous forms are usually present as forms of crystalline saccharides and that hydrous crystals can be converted into anhydrous crystals, and vice versa. The forms are mutually converted through a dehydration process performed by heating (drying method) and moisture absorption. Saccharides can be effectively used on an industrial scale by using those characteristics of converting the forms between hydrous and anhydrous crystal.

Carbohydrates have a wide range of applications in a variety of industries (textiles, plastics, food, pharmaceuticals, etc.) and are also used in biological applications. For example, lactose is commonly used as filler in pharmaceutical capsule and tablet formulations and as a carrier in dry powder inhalation devices (Chidavaenzi et al. 1997; Buckton et al. 2002). It is also commonly applied as an ingredient in dairy-based food products such as infant formula. The α-monohydrate is typically used in wet granulation formulations, while the β-anhydrous phase is used as a direct compression ingredient. For direct tableting, different forms of agglomerated lactose, such as spray-dried lactose, are used. The drying step in the manufacturing of spray-dried α-monohydrate is a very sensitive operation because of the possibility of physical modifications of lactose occurring during the drying process (Vromans et al. 1987).

This chapter investigates crystal transformation characteristics from the hydrate to the anhydrate via solvent-mediated transformation, in order to produce new crystalline material with an improved specific surface area. The kinetics and mechanism of the crystal transformation are discussed, as well as the influence on the crystal structure and possible applications.

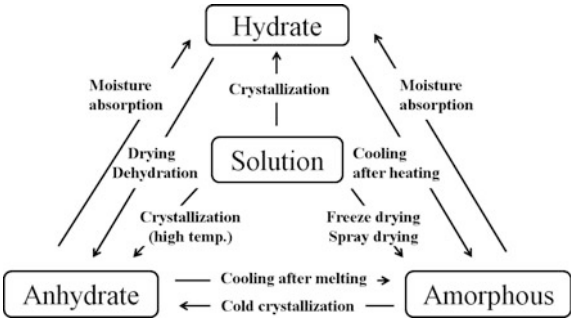

Fig. 8.1 Phase transitions of sugars

8.2 Phase Transitions of Carbohydrates

A phase transition is the transformation of a thermodynamic system from one phase or state of matter to another. The phases of a thermodynamic system and the states of matter have uniform physical properties. In the case of sugars, the possible phase transitions are summarized in Fig. 8.1. Starkting from a sugar solution, either the hydrate or the anhydrate crystalline forms can be obtained by crystallization under appropriate conditions. The anhydrate formation is typically favored by high-temperature conditions (Roos and Karel 1992; Figura and Epple 1995; Jones et al. 2006). The amorphous (i.e. non-crystalline form) phase can be produced by processes such as grinding (Lappalainen et al. 2006), freeze drying (Gabarra and Hartel 1998; Kedward et al. 1998; Gloria and Sievert 2001; Mazzobre et al. 2001), spray drying (Aguilar et al. 1994; Sussich and Cesàro 2008), lyophilization (Van Scoik and Carstensen 1990), by cooling after melting (Sussich et al. 2002), or dehydration and melt quenching (Surana et al. 2004; Simperler et al. 2006). The hydrate form is also produced from either the anhydrate or the amorphous phase by moisture absorption under mild or high relative humidity conditions (Crowe et al. 1996; Arvanitoyanis and Blanshard 1994; Kedward et al. 2000). The anhydrate phase can sometimes be obtained from the amorphous phase by cold crystallization (Saleki-Gerhardt and Zografi 1994) or by vacuum drying (O'Brien 1996). Note that the stability of the anhydrate crystal is an important parameter, which is studied in this dissertation. Finally, the transformation from hydrate to anhydrate is called dehydration (Jones et al. 2008; Kilburn and Sokol 2009). It is literally the removal of water from the hydrate to form the anhydrate.

8.3 "Soft" Dehydration and "Hard" Dehydration

Carbohydrate materials, especially mono- and disaccharides and products in which these are the main components such as fruit juices, are difficult to dry because of their sensitivity to temperature and water. In the food industry, it is the usual

procedure to detect and to use the most stable structure that consequently has the lowest solubility and the lowest free energy. Unfortunately, this is inconsistent with the demand for high dissolution rates because the phase with the lowest solubility in a certain solvent usually has the lowest dissolution rate.

In order to improve the dissolution rates, it is often necessary to lower the stability or to modify other physical properties of the compound. One of these physical properties is the shape of the crystals. Needle-like crystals lead to a significant increase in the specific surface area, thus improving the dissolution behavior and chemical reactions. Such an increase can be achieved by suspending crystalline substances in a solvent that induces phase transformations (Nordhoff and Ulrich 1999). In order to find new structures of several crystalline compounds, the methodology presented and applied in this chapter is devoted to the dehydration mechanism of molecular crystals and uses the existence of hydrates to promote the formation of new molecular packings by means of suitable dehydration conditions.

The simplified representation presented in Fig. 8.2 illustrates the fact that, starting from a hydrated structure, the use of hard dehydration conditions (such as high-temperature drying) is likely to produce an amorphous material, which can in turn evolve towards an anhydrous variety by means of a nucleation and growth mechanism. On the contrary, soft dehydration conditions (such as solvent-mediated crystal transformation) usually lead to a cooperative departure of water molecules, followed by a structural reorganization step leading to the nearest possible crystalline packing. This type of mechanism is, therefore, characterized by the persistence during the dehydration process of similar molecular contacts in the whole sample, in connection with the occurrence of continuous and cooperative molecular movements.

Moreover, the resulting packing is, at least to a certain extent, determined by the initial hydrated structure, so a part of the structural information is preserved, whereas the critical step for destructive–reconstructive mechanisms is likely to be the nucleation of the anhydrous variety, and the structural information is lost during such processes.

8.4 Solvent-Mediated Crystal Transformation Applied to Sugars

Trehalose, the disaccharide of α-D-glucose, is a naturally occurring sugar thought to confer anhydrobiotic properties (the ability to survive desiccation for long periods of time and rapidly resume metabolism upon rehydration) on various organisms in which it is found (Sussich et al. 2001, 2010). Trehalose shows polymorphism (Akao et al. 2001). In addition to the dihydrate, at least two anhydrous forms can be produced (Pinto et al. 2006). The anhydrous β-form can be formed from trehalose dihydrate by heating at 130 °C for 4 h (Reisener et al. 1962) or heating in a calorimeter under highly humid conditions, when it forms at 90 °C (Furuki et al. 2006). The anhydrous α-form is produced from the dihydrate by a slow thermal dehydration or supercritical CO_2 fluid extraction of the crystallization water

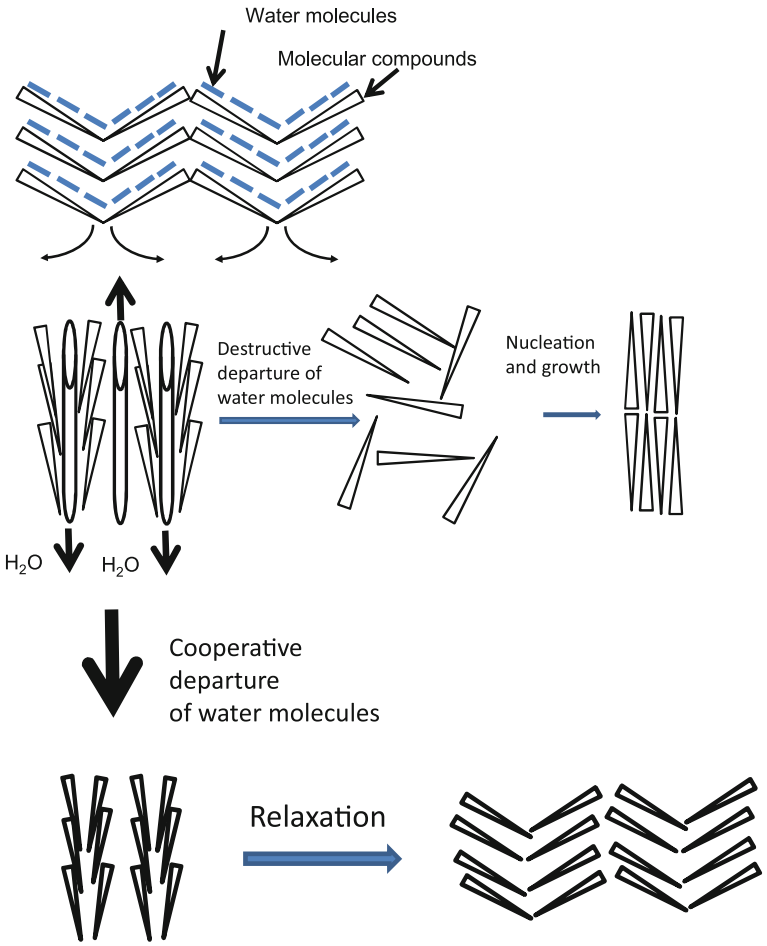

Fig. 8.2 Simplified representation of the methodology allowing the research of new polymorphic forms on the basis of different dehydration mechanisms (Garnier et al. 2002)

(Nagase et al. 2003; Willart et al. 2003). This anhydrous α-form can also be produced by dehydrating trehalose dihydrate at low relative humidity (0.1 % RH) and ambient temperature (25 °C) (Jones et al. 2006).

However, during these transformations, no drastic changes have been reported on the crystal structure.

Ohashi et al. (2007) investigated the crystal transformation of dihydrate trehalose to anhydrous trehalose using ethanol, and a new type of crystal particle with porous structure was obtained. The specific surface area of the anhydrous crystal transformed at 50 °C was 3.3 m^2/g, which was 5–8 times greater than that for anhydrous trehalose produced by vacuum drying and, for comparison, about 6 times higher than spray-dried α-lactose monohydrate (Cal et al. 1996) and 4–6

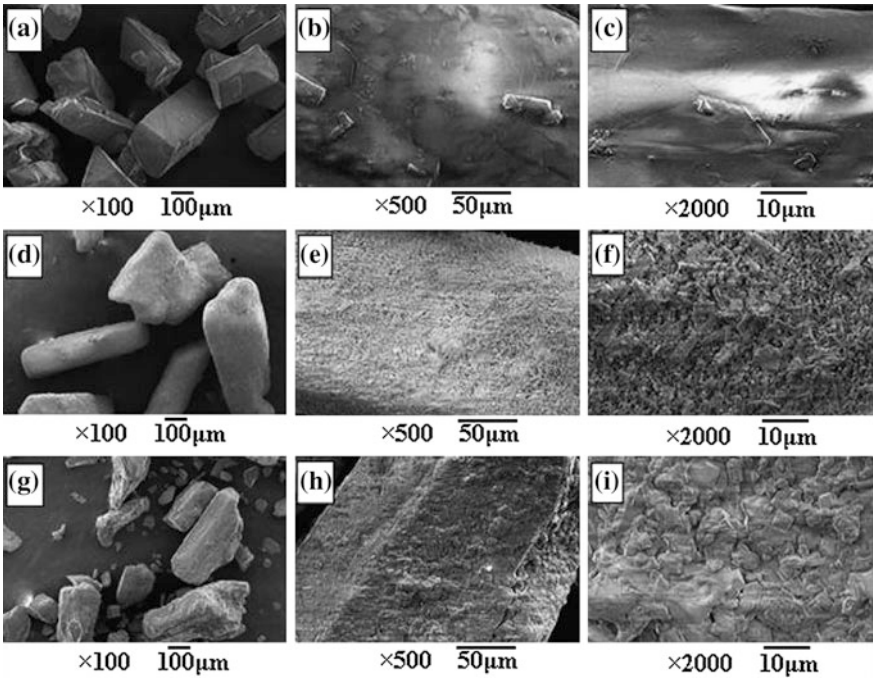

Fig. 8.3 SEM photomicrographs of anhydrous trehalose: **a–c** dihydrate crystal from solution, **a** ×100, **b** ×500, **c** ×2000, **d–f** anhydrous crystal transformed by the ethanol method, **d** ×100, **e** ×500, **f** ×2000, **g–i** anhydrous crystal transformed by vacuum drying, **g** ×100, **h** ×500, and **i** ×2000 (Ohashi et al. 2007)

times higher than some lactose excipients (Pitchayajittipong et al. 2010). The ethanol-mediated crystal transformation was performed in a glass reactor equipped with an agitation system. The porous structure of the anhydrous trehalose is shown in Fig. 8.3.

Garnier et al. (2002) observed the formation of whisker-like crystals during the dehydration of α-lactose monohydrate to anhydrous α-lactose in methanol or anhydrous β-lactose in acetone. Crystalline α-lactose monohydrate was obtained by Parrish and Brown (1982) from aqueous methanol or acetone solutions. The authors highlighted that the transformations were solid-state reactions, and not dissolution followed by recrystallization. In a recent work by Ohashi et al. (2009), α-cyclodextrin (α-CD) hexahydrate was transformed by the ethanol method, and porous crystalline ethanol dihydrate was obtained. The pore volume measured was 0.25 mL/g and the median pore diameter was 0.11 μm. The dissolution rate of this crystal was several times higher than that of (α-CD) hexahydrate or anhydrate obtained by drying.

8.5 Kinetics of Solvent-Mediated Crystal Transformation

For isothermal crystal transformation, the crystallization kinetics of many crystalline materials can be analyzed by the Avrami equation, which is generally written as:

$$x_c(t) = 1 - \exp(-(kt)^n) \tag{8.1}$$

In this equation, $x_c(t)$ is the normalized crystalline content at time t, and k and n are Avrami constants and are indicative of crystallization mechanisms that are involved. The exponent n in can provide information on nucleation type and crystal growth geometry. k is dependent upon the shape of the growing crystalline entities (e.g. whether they are spheres, discs, or rods), as well as the type and amount of nucleation (sporadic or predetermined). Basically, k is dependent on the reaction temperature, T_c, which affects the nucleation and growth rate. This equation is also called the Weibull distribution function, which has been successfully applied to describe shelf-life failure. A general empirical equation described by Weibull (1951) could be adapted to the dissolution/release process. This Avrami equation is essentially analogous to the equation of Kohlraush-Willian-Watt (KWW) (Alie 2004). Taking the logarithm of both sides of Eq. (8.1), we can get the parameter n as slope by plotting $\ln[-\ln(x_c)]$ versus $\ln t$ and release rate constant k from the interception at $\ln t = 0$. For n value, $n = 1$ represents the first-order reaction, and $n = 0.54$ represents the diffusion-limiting reaction kinetics. Table 8.1 shows the proposed equation for various solid reaction n values by Hancock and Sharp (1972). The derivation of the original model equation for solid reaction can be checked in the review by Hulbert (1969). Flavor release rates are strongly affected by the environmental humidity and temperature.

Figure 8.4 shows a typical endothermic peak of crystal transformation from hydrous sugar to its anhydrous form obtained from the isothermal measurement.

At constant temperature, the conversion fraction, $x(t)$, of the crystal transformation at time t is defined as:

$$x(t) = \frac{F(t)}{F(t_e)} \tag{8.2}$$

Table 8.1 Comparison of release mechanism n values using the model in the Avrami equation

Value of n	Release mechanism	Model equation
0.54	Diffusion-controlled (sphere)	$\left[1 - R^{1/3}\right]^2 = kt$
0.57	Diffusion-controlled (cylinder)	$R\ln R + 1 - R = kt$
0.62	Diffusion-controlled (tablet)	$(1 - R)^2 = kt$
1.00	First-order mechanism	$-\ln R = kt$
1.07	Moving interface mechanism (sphere)	$1 - R^{1/3} = kt$
1.11	Moving interface mechanism (disk)	$1 - R^{1/2} = kt$
1.24	Zero-order mechanism	$1 - R = kt$

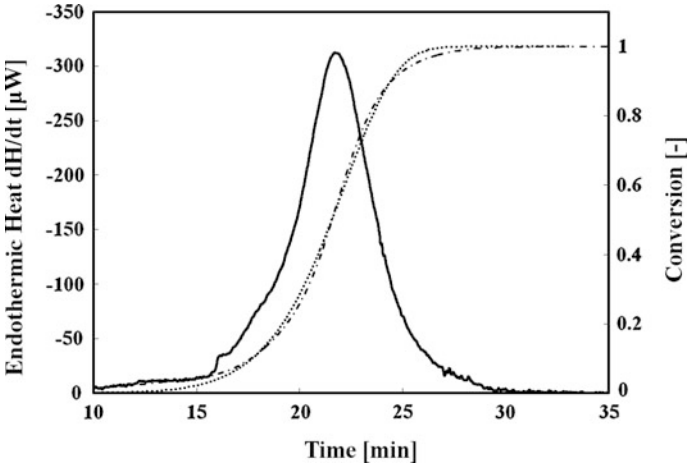

Fig. 8.4 DSC thermogram for crystal transformation from dihydrate trehalose to anhydrous trehalose under the isothermal conditions at 70 °C in ethanol with 2.5 % water content. *Solid line* Endothermic peak of crystal transformation; *Dashed line* conversion of the transformed material (using Simpson's rule); *Dotted line* conversion of the transformed material calculated with the Avrami equation for $n = 10$

where $F(t)$, as given by Eq. (8.2), is the integration of the DSC curve between the onset time of the crystal transformation reaction t_0 and an arbitrary time t before complete transformation is achieved at t_e, and $F(t_e)$ is the integration between t_0 and t_e, corresponding to the complete transformation.

$$F(t) = \int_{t_0}^{t} \left(-\frac{\mathrm{d}H}{\mathrm{d}t} \right) \mathrm{d}t \tag{8.3}$$

The term H represents the enthalpy of crystal transformation. The integration of Eq. (8.3) was approximated by numerical integration using Simpson's rule. The great number of data points makes this approximation highly accurate. For isothermal crystal transformation, the crystallization kinetics of many crystalline materials can be analyzed by the Avrami Eq. (8.1) (Avrami 1939, 1940).

For each T_c, the parameter n was fixed at 10, and the value of k that fitted best to the experimental data was calculated using Microsoft Excel Solver (dotted line in Fig. 8.4). The activation energy of the reactions was then approximated by the Arrhenius equation:

$$k = A \exp\left(-\frac{E_i}{RT_c} \right) \tag{8.4}$$

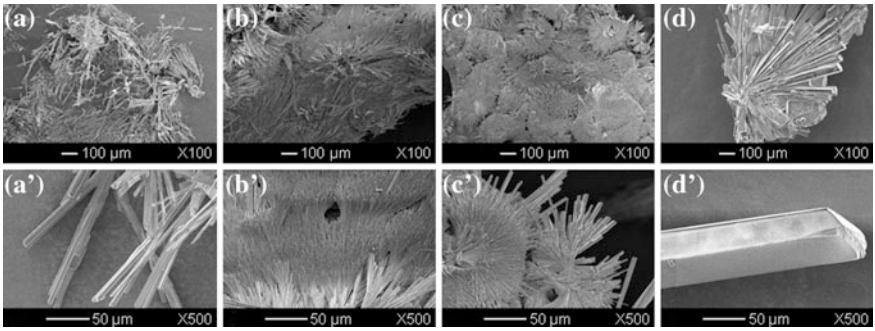

Fig. 8.5 Morphology of the anhydrous crystals obtained after a 30-min reaction at 70 °C with ethanol of **a**, **a′** 0.005, **b**, **b′** 0.4, **c**, **c′** 2.5, **d**, **d′** 4.0 % water content. The microscopy was performed at ×100 (**a**–**d**) and ×500 magnification (**a′**–**d′**)

where A is the frequency factor, E_i is the activation energy in J/mol, R is the gas constant (8.314 J/mol·K), and T_c is the reaction temperature in K. The activation energy was determined experimentally by carrying out the reaction at different reaction temperatures T_c.

8.6 Trehalose

Trehalose is a natural, non-reducing sugar formed from two glucose units joined by a 1-1 alpha bond, giving it the name of α-D-glucopyranosyl-(1 → 1)-α-D-glucopyranoside. Trehalose can be synthesized by fungi, plants, and invertebrate animals. It is implicated in anhydrobiosis, the ability of plants and animals to withstand prolonged periods of desiccation. It has high water retention capabilities and is used in food and cosmetics (Higashiyama 2002; Teramoto et al. 2008). There are two dominant theories as to how trehalose works within an organism in a state of cryptobiosis. They are the verification theory (Sussich et al. 2001), a state that prevents ice formation, and the water displacement theory (Crowe 1971), whereby water is replaced by trehalose, although it is possible that a combination of the two theories are at work (Sola-Penna and Meyer-Fernandes 1998). The only hydrous form of trehalose is dihydrate trehalose, but there are several anhydrous forms of trehalose. The first is known as the β-form and shows a characteristic melting point at 215 °C. The second is the α-form. Thermograms for this anhydrous α-form display a first endothermic peak at 125 °C and a melting slightly above 215 °C. The third anhydrous form, γ, is not stable, and can be distinguished from the α-form by the method of preparation, the enthalpy of melting, and the X-ray diffraction patterns (Sussich et al. 1998). Ohashi et al. (2008) investigated the encapsulation of flax seed oil in anhydrous crystalline maltose and trehalose with porous structure. Porous crystalline trehalose could be used as the matrix to encapsulate functional compounds. However, there are few investigations about the formation of anhydrous trehalose with porous structure by using solvent dehydration.

In the present study, the dehydration of trehalose in ethanol was performed in a differential scanning calorimeter in a high pressure crucible pan filled with ethanol.

Figure 8.5 shows the morphology of the anhydrous trehalose crystals obtained with ethanol of different water content after a 30 min transformation at 70 °C (isothermal conditions). The crystal size is highly dependent on the water content of the ethanol used. The crystals obtained with ethanol of 0.005 and 0.4 % water content had similar size and shape, with an average diameter of about 3 μm and an average length of 100 μm (Fig. 8.5a, b). When the water content in ethanol increased to 2.5 %, the size of the acicular crystals also increased, reaching an average diameter and length of 6 and 150 μm, respectively (Fig. 8.5c). At 4.0 % water content, the size of the acicular crystals greatly increased, achieving an average diameter and length of up to 50 and 500 μm, respectively (Fig. 8.5d).

Figure 8.6 shows the crystal transformation behavior from the initial dihydrate crystals to the anhydrous form. The dihydrate trehalose crystals surface is smooth and no porosity is observed (Fig. 8.6a). After 20 min of reaction at 70 °C in the ethanol of 0.005 % water content, cracks were observed on the surface of the crystals (Fig. 8.6b). At 25 min, slender acicular crystals were forming presumably from the surface to the core of the initial dihydrate crystal (Fig. 8.6c, d). These crystals are agglomerated in an acicular arrangement. Each needle has a diameter between 1 and 9 μm and an average length around 100 μm. The length-to-diameter ratio was over 10. The acicular crystals adopted a radial orientation. In these experiments, the anhydrous crystals obtained with the ethanol method were on average 10 times smaller than the dihydrate crystals. The needle-like crystal form can be attributed to the high local supersaturations during the dehydration phase (Nordhoff and Ulrich 1999). This property can be explained by the lower solubility of trehalose in pure ethanol (close to 0) than in water (0.509 g/g solution) (Bouchard et al. 2007). Assuming that the initial dihydrate crystals are squares with a diameter of 800 μm, the specific surface area increases by a factor of more than 150 if these squares are transferred into a number of smaller anhydrates crystals of

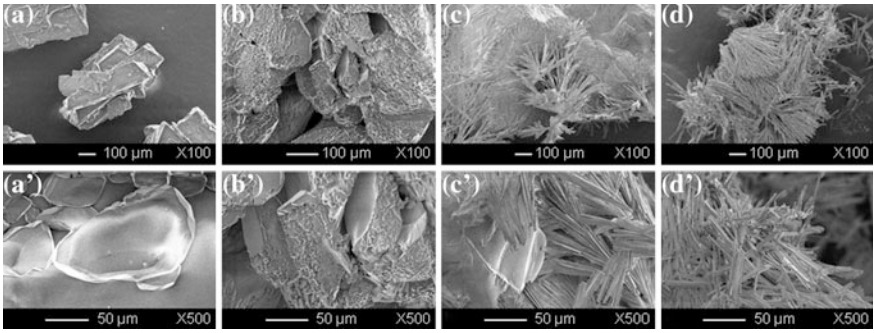

Fig. 8.6 Evolution of crystal structure through the isothermal crystal transformation at 70 °C using ethanol with 0.005 % water content. **a**, **a′** initial dihydrate trehalose; **b**, **b′** 20 min after reaction; **c**, **c′** 25 min after reaction; **d**, **d′** anhydrous trehalose obtained 30 min after reaction. The microscopy was performed at ×100 (**a**–**d**) and ×500 magnification (**a′**–**d′**)

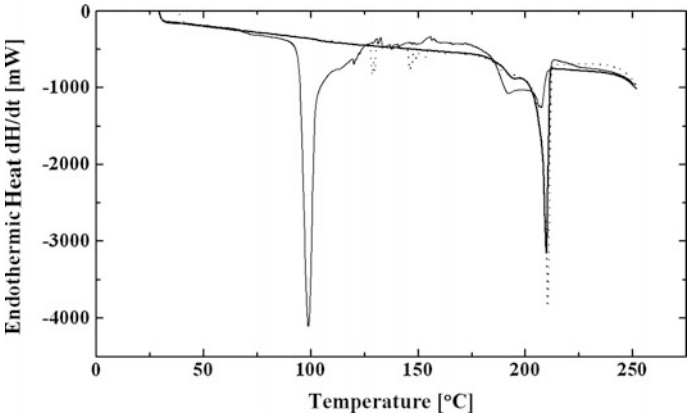

Fig. 8.7 Typical DSC thermographs of dihydrate trehalose (*thin line*), anhydrous trehalose obtained under isothermal (*thick line*) and non-isothermal conditions (*dashed line*)

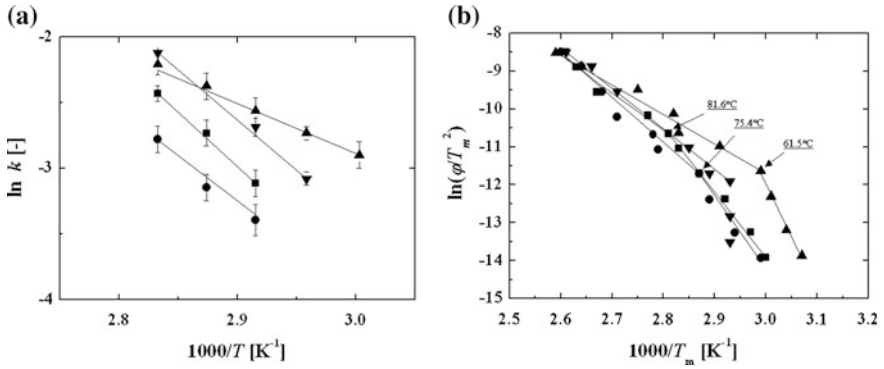

Fig. 8.8 Effect of the water content of ethanol on the crystal transformation kinetics under the **a** isothermal and **b** non-isothermal conditions (Kissinger's method). The water content of ethanol was 0.005 (*filled square*), 0.4 (*filled circle*), 2.5 (*upward pointing filled triangle*), and 4.0 % (*downward pointing filled triangle*)

100 μm in length and 3 μm in diameter. The increase in specific surface area is stronger if the diameter of the needle-like crystals is thinner.

Figure 8.7 shows the comparison between the DSC curves for the dihydrate and anhydrous trehalose. The dotted line represents the DSC curve of the anhydrous trehalose obtained under non-isothermal condition. The thermograph of the dihydrate trehalose revealed two peaks: a comparatively sharp peak at 100 °C, and a wider peak around 200 °C. The area of the endothermic peak of crystal transformation was approximated by numerical integration using Simpson's rule. The great number of data points makes this approximation highly accurate.

The crystallization kinetics of trehalose can be analyzed by the Avrami equation with 10 for the n parameter and the value of k which gave the best to the experimental data was calculated.

Table 8.2 Various properties of different β-maltose samples

Properties sample	Purity	β-form (%)	Degree of crystallinity (%)	DSC peak temperature (°C)	Crystallinity-corrected heat of fusion (J/g-crystal)
As-received Mβ-H_2O	99.3	96	91.0	131.6	191
$M\beta_s$	99.3	95	89.0	165.5	120
$M\beta_h$	99.3	93	84.8	132.4	73

$M\beta_s$ was obtained by ethanol-mediated transformation at 70 °C for 60 min, and $M\beta_h$ was obtained by vacuum drying at 56 °C for 4 days

Arrhenius plots of the transformation reaction rate constant, *k,* obtained under isothermal conditions are shown in Fig. 8.8. All displayed results correspond to an *n* value of 10. The activation energies of the crystal transformation E_i in ethanol of 0.005, 0.4, 2.5, and 4.0 % water content were determined to be 69, 62, 34, and 62 kJ/mol, respectively (Table 8.2). The activation energy at 2.5 % water content was only about half of that of the other treatments. In ethanol of 4.0 % water content, dehydration did not occur at temperatures below 65 °C.

The crystal transformation of dihydrate trehalose to anhydrous trehalose might be made up of two steps, which are crystal transformation from dihydrate trehalose to amorphous trehalose including the dehydration step and crystal growth from amorphous trehalose to anhydrous trehalose. The driving force of dehydration might be due to the higher chemical potential of water in the trehalose crystals.

We suppose that, for low temperature processes, the main phenomenon would be a diffusion of water of hydration of trehalose to the ethanol, increasing its water content. Moreover, the driving force of dehydration might be dependent on the water content in ethanol, and the diffusion coefficient of water in dihydrate trehalose might depend on the physical phase state of trehalose such as glassy state or rubbery state. Thus, the dehydration does not happen at low temperatures (glassy state) with 4.0 % water content in ethanol (lower driving force). The crystal growth rate might depend on the trehalose solubility in ethanol containing water. The increase in ethanol fraction causes a decrease in solubility of the dihydrate trehalose, has already been reported (Bouchard et al. 2007). Therefore, the water content of ethanol is the most important factor on this crystal transformation and affected the complex dependency of the crystal transformation rate.

8.7 Maltose

Maltose is arguably one of the most important sugars used in the food industry (Hodge et al. 1972, Odaka 1989). It is a disaccharide formed from two units of glucose joined with an α(1 → 4) bond. It is found in germinating seeds such as barley as they break down their starch to use for growth. Anhydrous maltose and maltose monohydrate are commercialized worldwide mostly as 90 % pure

crystalline product for the food industry. In addition, maltose is used in pharmaceuticals as a raw material, for example, in infusions in which it is sold as 99 % purity or above as a crystalline product. There are two anomers for maltose crystals: the α- and the β-forms, which have different crystal structures, melting points, solubility, and dissolution rates. In solution, one anomer can be converted into another by rearrangement of the position of the OH group in the open chain (anomerization). Maltose has three crystalline forms: anhydrous α-maltose (Quiqley et al. 1970), β-maltose monohydrate (Takusagawa and Jacobson 1978), and anhydrous β-maltose. Although they are anomers related via mutarotation, these forms can be regarded as polymorphs since there is a rapid inter-conversion between forms within solution. It is well recognized now that polymorphs, due to the difference in their potential energy levels, can have significant impact on the pharmaceutical behavior of a compound, for example, stability, solubility, and bioavailability.

In this study, the as-received maltose monohydrate (Mβ-H_2O) crystals were dehydrated using ethanol, and a new stable anhydrous β-maltose crystal form ($M\beta_s$) with fine pores was obtained. X-ray diffraction was used to characterize the crystalline difference from the anhydrous β-maltose obtained by vacuum drying ($M\beta_h$). In order to elucidate the reaction mechanism of the overall crystal transformation, the evolution of water content in the sample was measured. Thermal analysis was performed and the reaction kinetics was analyzed by the Jander and the Arrhenius equations. The evolution of the surface structure and anomer ratio was also investigated using scanning electron microscopy and gas chromatography.

The evolution of the crystal structure during crystal transformation at 50 °C is shown in Fig. 8.9. Before the reaction started, the as-received Mβ-H_2O crystals were present in a variety of shapes with sizes ranging from 100 to 300 μm and smooth surfaces. After 10 min of reaction time, some small pores were formed at the surface of the initial crystals. The number of pores increased as the reaction progressed. After 60 min of reaction, the crystal surface was covered with numerous needle-like crystals with a diameter below 1 μm and a maximum length of 10 μm. The crystal surface looked similar after 180 min of reaction and did not

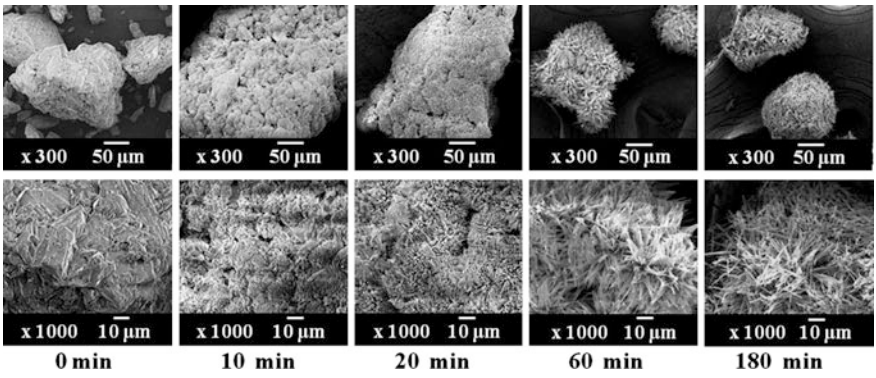

Fig. 8.9 Evolution of maltose crystal structure during crystal transformation in ethanol at 50 °C

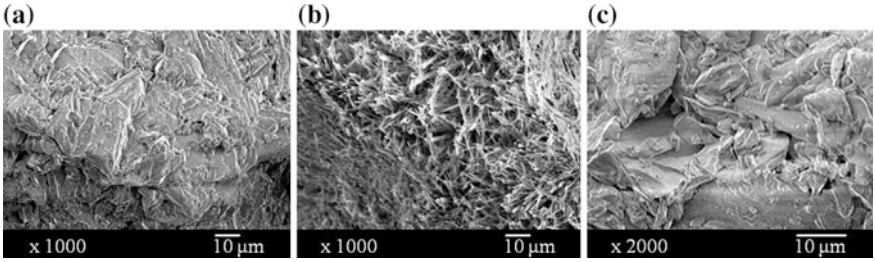

Fig. 8.10 Morphological comparison of **a** the as-received Mβ-H_2O, **b** $M\beta_s$, and **c** $M\beta_h$ crystals

seem to evolve more. The surface structures of three kinds of maltose crystals, the as-received Mβ-H_2O, $M\beta_s$, and $M\beta_h$ are shown in Fig. 8.10. $M\beta_s$ particles appeared to be porous aggregations of fine needle-like crystals. In contrast to $M\beta_s$, the as-received Mβ-H_2O and $M\beta_h$ both presented smooth surfaces.

The specific surface area of the porous $M\beta_s$, measured by the nitrogen adsorption isotherms, was 3.39 m^2/g, and those of the as-received Mβ-H_2O and $M\beta_h$ were 0.46 and 0.82 m^2/g, respectively. The specific surface area of $M\beta_s$ was about four- to sevenfold larger than those of the as-received Mβ-H_2O and $M\beta_h$. $M\beta_s$ also showed a relatively large intrusion volume (1.05 mL/g) and a median pore diameter of 1.26 µm, as measured by the mercury filling method (Ohashi 2010). The as-received Mβ-H_2O and $M\beta_h$ had much larger median pore diameters of 11.2 and 14.7 µm, respectively.

DSC profiles of the as-received Mβ-H_2O, $M\beta_s$, and $M\beta_h$ are illustrated in Fig. 8.11. $M\beta_s$ used was obtained by ethanol-mediated transformation at 70 °C for 60 min, and $M\beta_h$ was obtained by vacuum drying at 56 °C for 4 days. The as-received Mβ-H_2O presented a single endothermic peak at 131.6 °C (a), while $M\beta_h$ showed a unique peak at 132.4 °C (c). Although $M\beta_h$ is anhydrous, the similarity in melting point between these two crystalline forms revealed that $M\beta_h$ was actually metastable and would tend

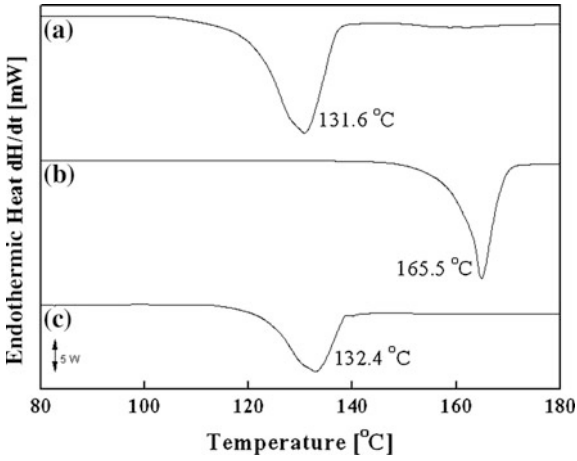

Fig. 8.11 Typical DSC profiles of **a** the as-received Mβ-H_2O, **b** $M\beta_s$, and **c** $M\beta_h$

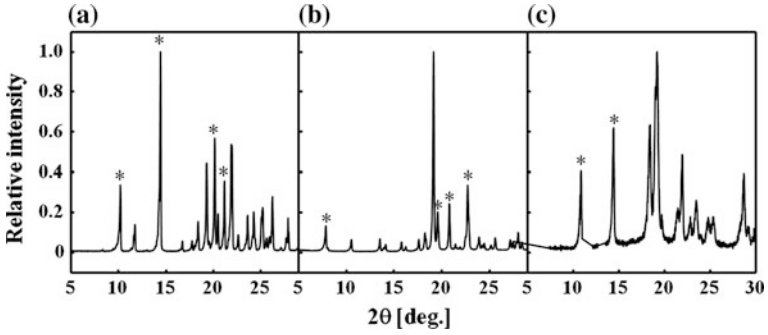

Fig. 8.12 Powder X-ray diffraction patterns of **a** the as-received Mβ-H_2O, **b** $M\beta_s$, and **c** $M\beta_h$. The symbol "*asterisk*" denotes a specific diffraction peak

to adsorb water to switch back to Mβ-H_2O. On the contrary, $M\beta_s$ (b) had a single peak at a much higher temperature (165.5 °C), which may be a clue of a higher stability. The as-received Mβ-H_2O crystals were of ca. 99.3 % purity, and the pure Mβ-H_2O was present mostly in the crystalline state (ca. 91 %), with a small fraction existing in the non-crystalline form (ca. 8.3 %). Table 8.1 shows the crystallinity-corrected heat of fusion of the three types of β-maltose crystals: the as-received Mβ-H_2O, $M\beta_s$, and $M\beta_h$. The uncorrected heat of fusion of the as-received Mβ-H_2O and $M\beta_s$ measured by DSC were 174 and 107 J/g of sample, respectively. The crystallinity-corrected heat of fusion of the β-form crystals were 191 and 120 J/g-crystal, respectively.

Powder X-ray diffraction of the as-received Mβ-H_2O (a), $M\beta_s$ (b), and $M\beta_h$ (c) are shown in Fig. 8.12. The as-received Mβ-H_2O showed characteristic diffraction peaks at 10.2°, 14.4°, 20.1°, and 21.2°. $M\beta_s$ had specific diffraction peaks at 7.9°, 19.2°, 20.8°, and 22.7°. $M\beta_h$ showed specific diffraction peaks at 10.9° and 14.8°. The results confirmed that $M\beta_s$ and $M\beta_h$ had completely different crystal forms. Kirk et al. (2007) and Crisp et al. (2010) have reported that the same characteristic powder X-ray diffraction pattern was generated from $L\alpha_s$ samples created by the dehydration of Lα-H_2O using both thermal (hard method) and solvent-mediated methods (soft method), indicating the same crystalline structure in both cases. Nonetheless, $M\beta_s$ was not obtainable by thermal treatment, but only via ethanol-mediated crystal transformation. The stable form of anhydrous β-maltose, $M\beta_s$ prepared in this paper would be advantageous for applications in the food and pharmaceutical industries, where thermodynamically stable crystalline forms are preferred for improvement of the shelf stability of products.

Figure 8.13 shows the time course of the moisture content of the maltose samples at different reaction temperatures (45, 50, 55, 60, and 70 °C). Dehydration is part of the crystal transformation process from Mβ-H_2O to anhydrous maltose. The solid lines are obtained by fitting Jander's equation to the experimental results. These good correlations suggested that the diffusion of water in maltose might be the rate-limiting process of this crystal transformation. The initial moisture content of the as-received Mβ-H_2O was 5.0 ± 0.1 %. The dehydration reaction was faster at higher temperatures. For 60 and 70 °C, the moisture content dropped to 0.5 %

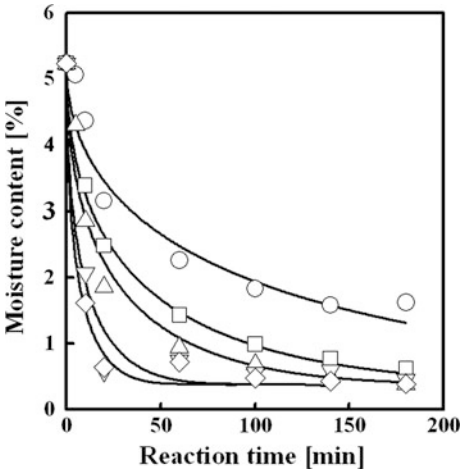

Fig. 8.13 Time courses of moisture content of β-maltose during ethanol-mediated crystal transformation at: 45 °C (*circle*), 50 °C (*square*), 55 °C (*upward pointing triangle*), 60 °C (*downward pointing triangle*), and 70 °C (*diamond*)

after 60 min of reaction. For 55 and 50 °C, the moisture content also decreased to 0.5 %, but a longer reaction time was needed (about 180 min). For the dehydration process at 45 °C, the dehydration reaction speed was the lowest, reaching a value of 1.6 % moisture content after 180 min and needed about 500 min to be completed. A minimum temperature of 50 °C was suitable to accomplish a complete transformation from Mβ-H_2O to $M\beta_s$ within 200 min.

In order to calculate the crystal conversion by the heat of fusion, the heat of fusion of both Mβ-H_2O and its dehydrated crystal, $M\beta_s$, are necessary to know. Figure 8.14 shows the time courses of the conversions determined by the disappearance of Mβ-H_2O (left) and the formation of $M\beta_s$ (right) with time at several temperatures. The conversions were calculated using the heat of fusion obtained by DSC measurements. Likewise, the solid lines are obtained by fitting Jander's equation to the empirical results. Modeling the conversion profiles using Jander's equation was satisfactory. The disappearance of Mβ-H_2O was roughly compensated by the formation of $M\beta_s$, indicating the conversion of Mβ-H_2O to $M\beta_s$. The apparent conversion rate constant increased with the increase in temperature. Mutarotation did not sufficiently occur during the crystal transformation of maltose by ethanol, as shown in Table 8.1. The as-received Mβ-H_2O contained 96 % β-form, and $M\beta_s$ contained 95 % β-form. This value is close to that obtained by Hodge et al. (1972) for $M\beta_h$ (93 % β-form). However, when placed in an environment at 57 % relative humidity (RH), the water content of $M\beta_h$ increased to 5.0 % after 2 h (initially 0.5 %), while $M\beta_s$ had a water content of 1.0 % after 100 h of storage at 57 % RH. $M\beta_s$ could presumably be considered a stable form of anhydrous β-maltose.

Jander's equation was also applied to the water loss in Mβ-H_2O to obtain the Arrhenius plots as illustrated in Fig. 8.15. The activation energy for the dehydration of Mβ-H_2O was found to be 100 kJ/mol, while the activation energy for the formation of $M\beta_s$ was 90 kJ/mol. The mechanism of the crystal transformation may consist of two steps. The first step would be the dehydration of Mβ-H_2O by ethanol and the dissolution to the amorphous state, which happened comparatively quite rapidly for higher

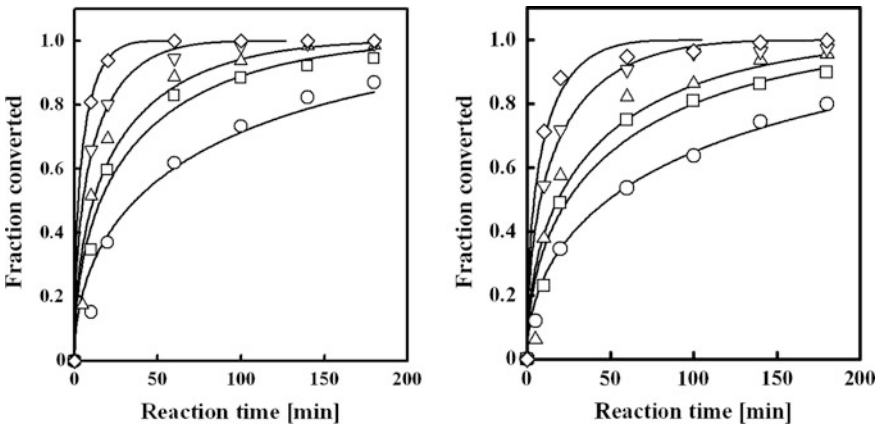

Fig. 8.14 Time courses of the conversions determined by the disappearance of Mβ-H_2O (*left*) and the formation of $M\beta_s$ (*right*) in ethanol at several temperatures. *Circle* 45 °C, *square* 50 °C, *upward pointing triangle* 55 °C, *downward pointing triangle* 60 °C, and *diamond* 70 °C. The *symbols* represent the calculated heats of fusion obtained by DSC measurements. The *solid lines* are the fittings of the empirical data by Jander's equation

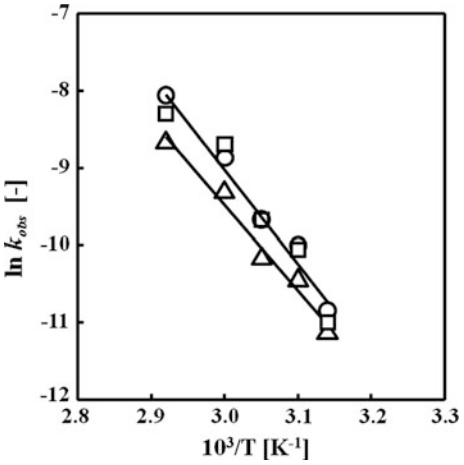

Fig. 8.15 Arrhenius plots of the conversion rates determined by the water loss in Mβ-H_2O (*circle*), the disappearance of Mβ-H_2O (DSC) (*square*), and the formation of $M\beta_s$ (DSC) (*diamond*)

temperatures. The second step would be the nucleation of $M\beta_s$ on the surface and its subsequent growth. This idea is confirmed by the variation of the conversion rates observed for a reaction carried out at 50 °C (Fig. 8.16). From this figure, it is understood that the conversion rate of the three reactions evolved the same way. However, the formation of $M\beta_s$ was not instant and seemed to proceed with a slight delay. This small lap of time would be necessary for the transition from the amorphous phase to the anhydrous crystalline form. The difference between the solubility of Mβ-H_2O (relatively high in ethanol containing a small amount of water) (Bouchard et al. 2007) and $M\beta_s$ drives the dissolution process and consequently determines the supersaturation

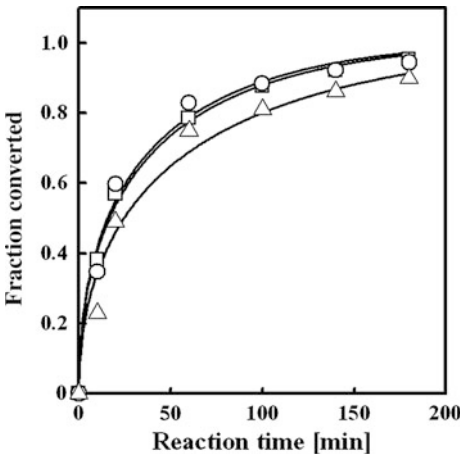

Fig. 8.16 Comparison of the conversions observed during the crystal transformation process carried out at 50 °C. The plots are the experimental data and the *solid lines* show the fittings of empirical data by Jander's equation. Water loss in the as-received Mβ-H_2O (*circle*). Disappearance of Mβ-H_2O (DSC) (*square*). Formation of $M\beta_s$ (DSC) (*diamond*)

level during the crystallization of $M\beta_s$. The ethanol-mediated crystal transformation of maltose might be dissolution controlled for lower temperatures, and crystallization controlled for higher temperatures, as indicated by the different activation energies for the dehydration and crystallization. Zhu et al. (1996) pointed out that in an anhydrate/hydrate system in mixtures of water and an organic solvent, water activity plays a crucial role. For a given temperature, there is an equilibrium value of water activity at which the solubility of the hydrate and the anhydrate are equal. The phase transformation from Mβ-H_2O to $M\beta_s$ may be the consequence of a deviation from the equilibrium water activity. The moisture content of ethanol is, therefore, a very important parameter of the crystal transformation. Anomerization of maltose is likely to happen in anhydrous polar solvents such as ethanol. Garnier et al. (2002) observed that the use of acetone as dehydrating solvent on single crystals of Lα-H_2O, without stirring, led to the unexpected formation of single crystals of the anomeric β-lactose (Lβ). The phenomenon was attributed to a complex mechanism, which requires minimum diffusion, so that mutarotation can occur before diffusion of water in acetone. Hence, Lβ was not obtained from a stirred suspension of Lα-H_2O in acetone. In ethanol, $L\alpha_s$ could be obtained from Lα-H_2O, probably via nucleation and growth processes. Since the solubility of $L\alpha_s$ is very low in ethanol (Bouchard et al. 2007), mutarotation would be unlikely in this solvent at room temperature. In the present ethanol-mediated crystal transformation of maltose, constant agitation assured sufficient diffusion preventing mutarotation at temperatures above 50 °C. As the reaction progressed, the moisture content of ethanol increased. This might be responsible for the reduction of $M\beta_s$ nucleation and growth rates because of the increasing supersaturation ratio.

Encapsulation of flax seed oil in crystalline maltose and trehalose with porous and non-porous structures was investigated to improve the stability of fatty acid during storage in the encapsulated oil (Ohashi et al. 2008). Anhydrous crystalline maltose and trehalose with a fine porous structure were obtained by dehydration using ethanol. The oil powders encapsulated with anhydrous crystalline maltose and trehalose with porous structure had good fluidity. The stability of fatty acids in

the encapsulated oil powders was investigated for storage at 40 °C. By using crystalline trehalose to encapsulate the fatty acids, degradation was markedly inhibited, irrespective of structure porosity. Formation of volatile aldehydes in porous crystalline trehalose or maltose was reduced to about half the amount of that for the non-porous crystalline encapsulates, indicating that volatile aldehyde was adsorbed into the fine pores.

8.8 Conclusion

In this chapter, the kinetics and mechanism of crystal transformation were discussed, as well as the influence on the crystal structure and the possible applications. The target compounds were mainly carbohydrates, including trehalose and maltose. Ethanol-mediated crystal transformation of sugars can lead to the formation of anhydrous porous crystal material with high specific surface area. These newly obtained sugar crystals have specific properties to form a creamy gel of sugar at supersaturated concentration. We can apply this porous sugar crystal to encapsulate flavor or functional food for the delivery of those compounds with release rate control.

References

Aguilar CA, Hollender R, Ziegler GR (1994) Sensory characteristics of milk chocolate with lactose from spray-dried milk powder. J Food Sci 59(6):1239–1243

Akao K, Okubo Y, Asakawa N, Inoue Y, Sakurai M (2001) Infrared spectroscopic study on the properties of the anhydrous form II of trehalose. Implications for the functional mechanism of trehalose as a biostabilizer. Carb Res 334:233–241

Alie J, Menegotto J, Cardon P, Duplaa H, Caron A, Lacabanne C, Bauer M (2004) Dielectric study of the molecular mobility and the isothermal crystallization kinetics of amorphous pharmaceutical drug substance. J Pharm Sci 93(1):218–233

Arvanitoyanis I, Blanshard J (1994) Rates of crystallization of dried lactose-sucrose mixtures. J Food Sci 59(1):197–205

Avrami M (1939) Kinetics of phase change I. J Chem Phys 7:1103–1112

Avrami M (1940) Kinetics of phase change II. J Chem Phys 8:212–224

Beckett ST, Francesconi MG, Geary PM, Mackenzie G, Maulny APE (2006) DSC study of sucrose melting. Carb Res 341:2591–2599

Boode K, Bisperink C, Walstra P (1991) Destabilization of O/W emulsions containing fat crystals by temperature cycling. Colloids Surf 61:55–74

Bouchard A, Hofland GW, Witkamp GJ (2007) Properties of sugar, polyol, and polysaccharide water-ethanol solutions. J Chem Eng Data 52:1838–1842

Buckton G, Chidavaenzi OC, Koosha F (2002) The effect of spray-drying feed temperature and subsequent crystallization conditions on the physical form of lactose. AAPS Pharm Sci Tech 3(4): Technical note 1

Cal S, Rodríguez-Puente B, Souto C, Concheiro A, Gómez-Amoza JL, Martínez-Pacheco R (1996) Comparison of a spray-dried α-lactose monohydrate with a fully hydrated roller-dried β-lactose. Int J Pharm 136:13–21

Carstensen T, Van Scoik K (1990) Amorphous-to-crystalline transformation of sucrose. Pharm Res 7:1278–1281

Chidavaenzi OC, Buckton G, Koosha F, Pathak R (1997) The use of thermal techniques to assess the impact of feed concentration on the amorphous content and polymorphic forms present in spray dried lactose. Int J Pharm 159:67–74

Christensen KL, Pedersen GP, Kristensen HG (2002) Physical stability of redispersible dry emulsions containing amorphous sucrose. Eur J Pharm Biopharm 53:147–153

Crisp JL, Dann SE, Edgar M, Blatchford CG (2010) The effect of particle size on the dehydration/rehydration behaviour of lactose. Int J Pharm 391:38–47

Crowe JH (1971) Anhydrobiosis: an unsolved problem. Am Nat 105:563–573

Crowe LM, Reid DS, Crowe JH (1996) Is trehalose special for preserving dry biomaterials? Biophys J 71:2087–2093

Devlin TM (ed) (2002) Amino acid metabolism, chapter no 18 of textbook of biochemistry with clinical correlations, 5th edn, pp 784–790. Wiley-Liss, New York

Figura LO, Epple MJ (1995) Anhydrous α-lactose. A study with DSC and TXRD. J Thermal Anal 44:45–53

Furuki T, Abe R, Kawaji H, Atake K, Sakurai M (2006) Thermodynamic functions of α, α-trehalose dihydrate and of α, β-trehalose monohydrate at temperatures from 13 K to 300 K. J Chem Thermodyn 38:1612–1619

Garnier S, Petit S, Coquerel G (2002) Dehydration mechanism and crystallinsation behaviour of lactose. J Therm Anal Cal 68:489–502

Gloria H, Sievert D (2001) Changes in the physical state of sucrose during dark chocolate processing. J Agric Food Chem 49:2433–2436

Grön H, Borissova A, Roberts KJ (2003a) In-process ATR-FTIR spectroscopy for closed-loop supersaturation control of a batch crystallizer producing monosodium glutamate crystals of defined size. Ind Eng Chem Res 42:198–206

Grön H, Mougin P, Thomas A, White G, Wilkinson D (2003b) Dynamic in-process examination of particle size and crystallographic form under defined conditions of reactant supersaturation as associated with the batch crystallization of monosodium glutamate from aqueous solution. Ind Eng Chem Res 42:4888–4898

Gabarra P, Hartel W (1998) Corn syrup solids and their saccharide fractions affect crystallization of amorphous sucrose. J Food Sci 63:523–528

Hammond RB, Lai X, Roberts KJ, Thomas A, White G (2004) Application of in-process X-ray powder diffraction for the identification of polymorphic forms during batch crystallisation reactions. Cryst Growth Design 4:943–948

Hancock JD, Sharp JH (1972) Method of comparing solid-state kinetic data and its application to the decomposition of kaolinite, brucite, and $BaCO_3$. J Am Cera Soc 55:74–77

Hartel Ri W, Shastry AV (1991) Sugar crystallization in food products. Crit Rev Food sci Nutr 30(1):49–112

Higashiyama T (2002) Novel functions and applications of trehalose. Pure Appl Chem 74 (7):1263–1269

Hodge JE, Rendleman JA, Nelson EC (1972) Useful properties of maltose. Cereal Sci Today 17:180–188

Hulbert SF (1969) Models for solid state reactions in powdered compacts. J Br Ceram Soc 6:11–20

Hurtta M, Pitkanen I, Knuutinen J (2004) Melting behaviour of D-sucrose, D-glucose and D-fructose. Carb Res 339:2267–2273

Jander WZ (1927) Reactions in the solid state at high temperatures I. Rate of reaction for an endothermic change. Anorg U Allgem Chem 163:1–30

Jones MD, Beezer AE, Buckton G (2008) Determination of outer layer and bulk dehydration kinetics of trehalose dihydrate using atomic force microscopy, gravimetric vapour sorption and near infrared spectroscopy. J Pharm Sci 97:4404–4415

Jones MD, Hooton JC, Dawson ML, Ferrie AR, Price R (2006) Dehydration of trehalose dihydrate at low relative humidity and ambient temperature. Int J Pharm 313:87–98

Kedward CJ, Macnaughtan W, Blanshard JMV, Mitchell JR (1998) Crystallization kinetics of lactose and sucrose based on isothermal differential scanning calorimetry. J Food Sci 63(2):192–197

Kedward CJ, Macnaughtan W, Mitchell JR (2000) Crystallization kinetics of amorphous lactose as a function of moisture content using isothermal differential scanning calorimetry. J Food Sci 65 (2):324–328

Kilburn D, Sokol PE (2009) Structural evolution of the dihydrate to anhydrate crystalline transition of trehalose as measured by wide-angle X-ray scattering. J Phys Chem B 113:2201–2206

Kimmerling ZJ (1984) An attempt to investigate changes of impurity concentration with the depth of crystal matrix of VHP sugar. Proc Congr S Afr Sugar Technol Assoc 58:51–53

Kirk JH, Dann SE, Blatchford CG (2007) Lactose: a definitive guide to polymorph determination. Int J Pharm 334:103–114

Kissinger HE (1957) Reaction kinetics in differential thermal analysis. Anal Chem 29:1702–1706

Lappalainen M, Pitkänen I, Harjunen P (2006) Quantification of low levels of amorphous content in sucrose by hyperDSC. Int J Pharm 307:150–155

Maruyama S, Ooshima H (2001) Mechanism of the solvent-mediated transformation of taltirelin polymorphs promoted by methanol. Chem Eng J 81:1–7

Mathlouthi M, Cholli AL, Koenig JL (1986) Spectroscopic study of the structure of sucrose in the amorphous state and in aqueous solution. Carb Res 147:1–9

Mazzobre MF, Soto G, Aguilera JM, Buera MP (2001) Crystallization kinetics of lactose in sytems co-lyofilized with trehalose. Analysis by differential scanning calorimetry. Food Res Int 34:903–911

Nagase H, Endo T, Ueda H, Nagai T (2003) Influence of dry conditions on dehydration of α, α-trehalose dihydrate. STP Pharma Sci. 13:269–275

Nakai Y, Yamamoto K, Terada K, Sasaki I (1986) Crystallization of amorphous α-, β-, γ-cyclodextrin by water sorption. Yakugaku Zasshi 106:420–424

Nordhoff S, Ulrich J (1999) Solvent-induced phase transformations of hydrates. J Therm Anal Cal 57:181–192

O'Brien J (1996) Stability of trehalose, sucrose and glucose to nonenzymatic browning in model systems. J Food Sci 61:679–682

Odaka E (1989) Anhydrous maltose. New Food Indus (in japanese) 17–22

Ohashi T (2010) Porous crystalline saccharide, its preparation and uses. U.S. patent 0222569 A1

Ohashi T, Yoshii H, Furuta T (2007) Innovative crystal transformation of dihydrate trehalose to anhydrous trehalose using ethanol. Carb Res 342:819–825

Ohashi T, Shibuya T, Oku K, Yoshii H, Furuta T (2008) Encapsulation of flax seed oil in anhydrous crystalline maltose and trehalose with porous structure (in Japanese). Nippon Shokuhin Kagaku Kogaku Kaishi. 55(1):13–17

Ohashi T, Verhoeven N, Okuda D, Furuta T, Yoshii H (2009) Formation of porous α-CD ethanol dihydrate by crystal transformation method. Eur Food Res Technol 230:195–199

Okuno M, Kishihara S, Otsuka M, Fujii S, Kawasaki K (2003) Variability of melting behavior of commercial granulated sugar measured by differential scanning calorimetry. Int Sugar J 105:29–35

Orford PD, Paker R, Ring SG (1990) Aspects of the glass transition behaviour of mixtures of carbohydrates of low molecular weight. Carb Res 196:11–18

Parrish MW, Brown ML (1982) Solid state transformations of α-Lactose monohydrate in alcoholic media. J Dairy Sci 65:1688–1691

Peica N, Lehene C, Leopold N, Schlucker S, Kiefer W (2007) Monosodium glutamate in its anhydrous and monohydrate form: differentiation by Raman spectroscopies and density functional calculations. Spectrochim Acta Part A 66:604–615

Pinto SS, Diogo HP, Moura-Ramos JJ (2006) Crystalline anhydrous α,α-trehalose (polymorph β) and crystalline dihydrate α,α-trehalose: a calorimetric study. J Chem Thermodyn 38: 1130–1138

Pitchayajittipong C, Price R, Shur J, Kaerger JS, Edge S (2010) Characterisation and functionality of inhalation anhydrous lactose. Int J Pharm 390:134–141

Quigley GJ, Sarko A, Marchessault RH (1970) Crystal and molecular structure of maltose monohydrate. J Am Chem Soc 92(20):5834–5839

Reisener HJ, Goldschmid HR, Ledingham GA, Perlin AS (1962) Formation of trehalose and polyols by wheat stem rust (*Puccinia graminis tritici*) uredospores. Can J Biochem Physiol 40:1248–1251

Rousseau D (2000) Fat crystals and emulsion stability—a review. Food Res Int 33(1):3–14

Roos Y, Karel M (1992) Crystallization of amorphous lactose. J Food Sci 57:775–777

Roos Y (1993) Melting and glass transitions of low molecular weight carbohydrates. Carb Res 238:39–48

Saleki-Gerhardt A, Zografi G (1994) Non-isothermal and isothermal crystallization of sucrose from the amorphous state. Pharm Res 11(8):1166–1173

Simperler A, Kornherr A, Chopra R, Bonnet PA, Jones W, Motherwell WDS, Zifferer G (2006) Glass transition temperature of glucose, sucrose, and trehalose: An experimental and in silico study. J Phys Chem B 110:19678–19684

Sola-Penna M, Meyer-Fernandes JR (1998) Stabilization against thermal inactivation promoted by sugars on enzyme structure and function: why is trehalose more effective than other sugars? Arch Biochem Biophys 360(1):10–14

Stryer L (ed) (1988). Biochemistry, 3rd edn, pp 18, 496, 579, 1026, Freeman, W.H, New York

Supratim G, Rousseau D (2011) Fat crystals and water-in-oil emulsion stability. Curr Opin Coll 16(5):421–431

Surana R, Pyne A, Suryanarayanan R (2004) Effect of preparation method on physical properties of amorphous trehalose. Pharm Res 21(7):1167–1176

Sussich F, Bortoluzzi S, Cesàro A (2002) Trehalose dehydration under confined conditions. Therm Acta 391:137–150

Sussich F, Cesàro A (2008) Trehalose amorphization and recrystallization. Carb Res 343:2667–2674

Sussich F, Skopec CE, Brady JW, Cesàro A (2001) Reversible dehydration of trehalose and anhydrobiosis: from solution state to an exotic crystal? Carbohydr Res 334:165–176

Sussich F, Skopec CE, Brady JW, Cesàro A (2010) Water mobility in the dehydration of crystalline trehalose. Food Chem 122:388–393

Sussich F, Urbani R, Princivalle F, Cesàro A (1998) Polymorphic amorphous and crystalline forms of trehalose. J Am Chem Soc 120:7893–7899

Takusagawa F, Jacobson RA (1978) The crystal and molecular structure of α-maltose. Acta Cryst B 34:213–218

Teramoto N, Sachinvala ND, Shibata M (2008) Trehalose and trehalose-based polymers for environmentally benign, biocompatible and bioactive materials. Molecules 13:1773–1816

Threlfall T (1995) Analysis of organic polymorphs. A review. Analyst 120:2435–2460

Urbani R, Sussich F, Prejac S, Cesaro A (1997) Enthalpy relaxation and glass transition behaviour of sucrose by static and dynamic DSC. Therm Acta 304(305):359–367

Vanhal I, Blond G (1999) Impact of melting conditions of sucrose on its glass transition temperature. J Agri Food Chem 47:4285–4290

Van Scoik KG, Carstensen JT (1990) Nucleation phenomena in amorphous sucrose systems. Int J Pharm 58:185–196

Vromans H, Bolhuis GK, Lerk CF, Kus-sendrager KD (1987) Studies on tableting properties of lactose VIII. The effect of variations in primary particle size, percentage of amorphous lactose and addition of a disintegrant on the disintegration of spray-dried lactose tablets. Int J Pharm 39:201–206

Weibull W (1951) A statistical distribution function of wide applicability. J Appl Mech 18:293–297

Willart JF, Danede F, De Gusseme A, Descamps M, Neves C (2003) Origin of the dual structural transformation of trehalose dihydrate upon dehydration. J Phys Chem B 107:11158–11162

Zhu H, Yuen C, Grant DJW (1996) Influence of water activity in organic solvent + water mixtures on the nature of the crystallizing drug phase. I Theophylline Int J Pharm 135:151–160

Chapter 9
Freezing and Freeze-Drying

Yrjö H. Roos, Caroline Furlong and Naritchaya Potes

Abstract Freezing of biological materials is commonly carried out to delay biochemical and chemical changes resulting in loss of activity. However, the physicochemical background of freezing is not well understood. Freeze-drying is generally aimed at preserving biological materials and bioactive components for long periods of time without the need for frozen storage. Freeze-drying relies on proper freezing and process control based on an understanding of the frozen state properties of biological materials. Such properties relate to unfrozen water and its distribution across carbohydrates, proteins and other components. Here, novel methods for deriving accurate protein hydration levels and unfrozen water content are discussed. The role of water and carbohydrate distribution in preserving the viability of micro-organisms in freezing and freeze-drying is also discussed, as this is a significant factor affecting the success of freezing and freeze-drying processes in the production of starter cultures and the preservation of probiotic bacteria.

Keywords Freezing · Freeze-drying · Glass transition · Carbohydrates · Proteins · Probiotic bacteria · Unfrozen water · Water activity

9.1 Introduction

Freezing of water is a complex process of ice formation at low temperatures, and is the fundamental principle of bioactive stabilization using freezing and freeze-drying. Freezing and frozen storage prior to freeze-drying is a process of microstructure formation for stability control of frozen and freeze-dried materials. In long-term stabilization, frozen materials need to be stored at conditions where unfrozen solutes remain in a vitrified solid state, while the vitrified solid state of frozen structures is preserved for long-term ambient storage by ice sublimation in the freeze-drying (lyophilization) process. The frozen material shows an expansion corresponding to the volume expansion of the same quantity of water converted to ice during crystallization.

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Protection of protein conformation and retention of biological functions subsequent to freezing and thawing or freeze-drying and rehydration is common in the food and pharmaceutical industries. The use of carbohydrates, particularly monosaccharides and disaccharides, as cryoprotectants to retain protein structures against freezing and dehydration damages has been of particular interest (Jorgensen et al. 2009). The phase behavior of water and the state of dissolved substances dominate the thermal properties of cryoprotectant systems. Ice formation always results in separation of a freeze-concentrated unfrozen aqueous cryoprotectant phase and vitrification of the maximally freeze-concentrated unfrozen phase over a cryoprotectant-specific temperature range (Roos and Karel 1991a, b; Luyet and Rasmussen 1968; Franks et al. 1977; Levine and Slade 1986; Pehkonen et al. 2008). The phase and state behavior of cryoprotectant–water systems may be described using state diagrams (Franks et al. 1977; Roos and Karel 1991a, b; Buera et al. 2011). While such diagrams have clarified the physicochemical properties of carbohydrate systems at low temperatures, they lack the information needed to understand carbohydrate–protein interactions in biological, food and pharmaceutical systems as well as intracellular and extracellular ice formation in cellular structures.

Although freezing and freeze-drying are well-established processes for preservation of biological materials, the present chapter highlights physicochemical properties of typical food and related materials affecting structure formation and properties of frozen and freeze-dried materials, with a particular focus on stabilization of bioactives.

9.2 Freezing and the Frozen State

The freezing of food materials is a well-established industry, as freezing stops or significantly delays microbial growth, and delays most biological processes and chemical reactions. Equilibrium freezing (referring here to ice formation and phase and state transitions below 0 °C) properties of eutectic salt solutions such as sodium chloride are well described in general physical chemistry (Atkins and De Paula 2010), but properties of sugar solutions and more complex dispersed structures are often poorly understood. Biological and food materials always contain unfrozen water at low temperatures. The term "unfrozen water" here refers to water in which interactions with solutes occur, although it is worth noting that the properties of even unfrozen pure water at temperatures within normal limits for supercooling and vitrification, i.e., −40 °C to −130 °C (*no man's land*), are poorly understood (Angell 2008, 2014). On the other hand, freezing and frozen storage of biological materials is routinely carried out using liquid nitrogen (liquid-N_2, −196 °C) and −80 °C equipment. The use of such temperatures is based on the freezing medium and equipment availability rather than scientific data for optimal material stability.

$$p_w = xp_0 \tag{9.1}$$

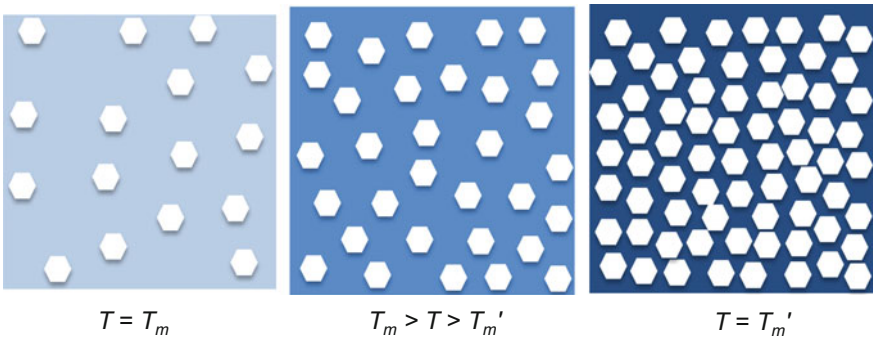

Fig. 9.1 Relative quantity of ice forming in biological materials at temperatures corresponding to equilibrium melting temperature, T_m; temperatures above the onset of ice melting in a maximally freeze-concentrated state, T_m'; and in a maximally freeze-concentrated state at T_m'

The freezing temperature depression of water by solutes in dilute solutions is well known, and is described by Raoult's law (Eq. 9.1), which states that the smaller the solute molecules, the larger their effect on water vapor pressure, p_w. The equilibrium melting temperature of the last ice crystals during heating of a solution with ice (i.e., freezing temperature), T_m, has the requirement of $p_i = p_w$ (where p_i is the vapor pressure of the ice). The T_m decreases with solute concentration, which for frozen systems implies that the amount of frozen water (ice) increases with decreasing temperature. Conversely, during frozen storage, temperature fluctuations can result in substantial melting–refreezing phenomena and rapid recrystallization of ice, with a consequent increase in the size of ice crystals. The temperature dependence of ice in frozen solutions is shown in Fig. 9.1. In a binary eutectic system, both the solvent water and the dissolved substance crystallize as solubility is exceeded, causing precipitation of the solute at the eutectic temperature, T_e. Eutectic freezing is unusual in complex multicomponent systems, and many biological materials show vitrification of their unfrozen water–solute phase. The glass transition temperature of the maximally freeze-concentrated unfrozen phase is known as T_g'. Ice formation in a maximally freeze-concentrated solute phase ceases at T_m' as the solute concentration approaches C_g'. A maximally freeze-concentrated material, therefore, shows an onset of the glass transition at T_g' and onset of ice melting at T_m' when measured in heating after freezing to the maximally freeze-concentrated state.

9.3 Glass Transition of Frozen Materials

Glass transition is a reversible change in the state of supercooled noncrystalline materials. The glass transition can be determined from thermodynamic properties such as heat capacity and thermal expansion of supercooled liquids as they are

heated and cooled over the glass transition temperature range (Angell 2002; Roos and Drusch 2015). Supercooled states of materials show molecular disorder and random time-dependent characteristics. The glass transition is accompanied by changes in mechanical and dielectric properties as well as other structural relaxations resulting from a change in the molecular mobility of the glass-forming substance. The structural relaxations occurring over the reversible glass transition are referred to as α-*relaxation*. The α-relaxation is typically studied in heating by monitoring the time and temperature dependence of the molecular response of the material during glass transition. The relaxation time is derived from the time dependence of the α-relaxation. The characteristic relaxation time, τ, for the solid, glassy state is 100 s, corresponding to a viscosity of 10^{12} Pa s (Angell 2002).

9.4 Carbohydrate Systems

Carbohydrates show a large variation in molecular size as components of biological materials. On the other hand, small sugars are often highly soluble and occur in large quantities in biological materials important to freezing and freeze-drying. Conversely, small sugars are the most common cryoprotectants used for preservation of starter cultures and native protein structures. Sugar systems have been studied in detail for understanding the frozen state phenomena of aqueous solutions of carbohydrates and cryoprotectant systems (Roos 1993; Corti et al. 2010; Roos and Drusch 2015).

Freezing of carbohydrate systems commonly occurs without solute crystallization, as described in Fig. 9.1. Solute crystallization is limited by the high solubility of many carbohydrates and their slow nucleation and crystal growth rates compared to that of ice formation. As a consequence, maximally freeze-concentrated states of carbohydrates are common, and are typically a prerequisite for successful freeze-drying where

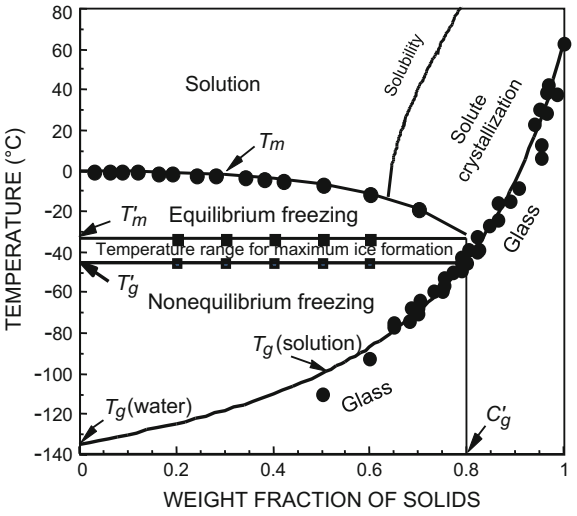

Fig. 9.2 State diagram of sucrose showing water content dependence of glass transition temperature, T_g; ice melting temperature, T_m; onset of glass transition of maximally freeze-concentrated solute phase, T_g'; and onset of ice melting in a maximally freeze-concentrated solution, T_m'

any liquid water must be part of the maximally freeze-concentrated vitrifying solute phase at temperatures lower than T_m'.

State diagrams are often constructed for binary carbohydrate–water systems to describe ice formation as well as freezing and freeze-drying properties of carbohydrate systems. The state diagram of sucrose is shown in Fig. 9.2.

9.5 Carbohydrate–Protein Systems

Protection of proteins and peptides against dehydration or freezing damage advances the survival of living organisms as well as the production and stabilization of active enzymes, pharmaceuticals and biological materials. Proteins contain hydration water in cavities and surface pores. Strong hydrogen bonding reduces the chemical potential of water molecules at hydration sites and explains the unfrozen state of hydration water at low temperature (Jansson et al. 2011; Roos and Potes 2015; Swenson and Cerveny 2015). The α-relaxation of hydration water in proteins is coupled with vitrification phenomena in cryoprotection and anhydrobiosis, particularly with the protection of protein conformation when carbohydrate protectants are used. Water molecules in amorphous carbohydrate systems retain high mobility, and they may exchange with water molecules that are hydrogen-bonded to the protein. Depending on the carbohydrate, vitrification of the hydration water and aqueous phase may occur at different temperatures, with consequent effects on protein stability (Roos and Potes 2015). In a protein–carbohydrate system, an exact quantity of water is required to exist as protein hydration water to satisfy a thermodynamic balance with a co-existing carbohydrate phase. At equilibrium, such hydration water has equal water activity a_w (equal chemical potential and vapor pressure) to that of water associated with the carbohydrate. Our studies have shown that the sugars co-existing with proteins in a freeze-concentrated solute phase give a T_g' for the unfrozen carbohydrate–unfrozen water phase. Measurement of the T_g' can be used to derive the composition of the unfrozen carbohydrate phase, while the unfrozen protein hydration water can be obtained from the water sorption data (Roos and Potes 2015). Protein hydration water in a maximally freeze-concentrated protein–carbohydrate system showed a quantity that became independent of the initial water content and the carbohydrate content, i.e., maximally freeze-concentrated carbohydrate was plasticized by a measurable unfrozen water fraction. The surrounding protein was maximally freeze-concentrated with a corresponding unfrozen hydration water fraction. A system composed of bovine whey proteins and a glucose–fructose (1:1) carbohydrate phase (GF) showed unfrozen water fractions in the maximally freeze-concentrated state with 0.164 g H_2O/g protein and 0.273 g H_2O/g GF. The data measured by Roos and Potes (2015) were used to establish a tertiary three-component state diagram shown in Fig. 9.3. The experimental quantification of the unfrozen water in the protein and carbohydrate components of cryoprotectant systems at maximum freeze-concentration as described by Roos and Potes (2015) provided a significant universal method of quantifying (i) protein hydration water and (ii) the unfrozen water in the carbohydrate phase. This

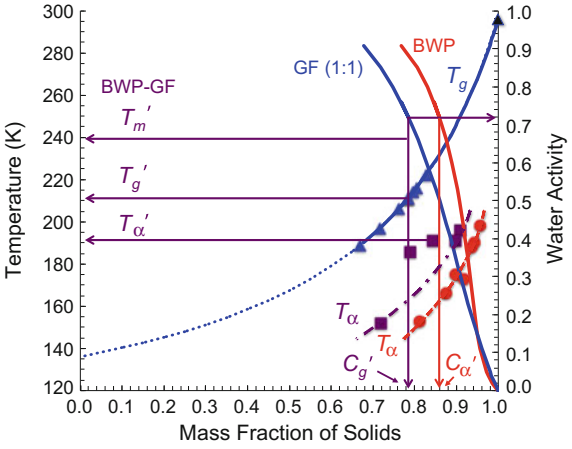

Fig. 9.3 Tertiary state diagram for bovine whey proteins (BWP) and glucose:fructose (1:1) as carbohydrates (GF). The glass transition of the GF, T_g, is found using differential scanning calorimetry at the temperature dominated by the GF phase. At maximum freeze-concentration, the glass transition, T_g', is that of the carbohydrate, while hydration water on protein shows an α-relaxation at T_α'. The water content corresponding to the glass transition of the carbohydrate, T_g, and α-relaxation of protein hydration water, T_α, can be derived from the component water sorption isotherms

is important for the understanding and use of carbohydrate protectants in freezing, lyophilization and dehydration of biological materials, and in the stabilization of bioactive components in formulation and structure engineering of food and pharmaceutical materials. It should be noted that earlier studies of protein hydration water have used a rough estimate of 0.2–0.3 g of unfrozen water/g of protein. Such estimates were made on the basis of unfrozen water measurements using differential scanning calorimetry (DSC) (Doster et al. 1986).

Roos and Potes (2015) showed that a separate α-relaxation of the protein hydration water and of the bulk GF were signs that hydration water molecules were primarily hydrogen-bonded to protein molecules, and that hydration water provided a bridge for hydrogen bonding of the protein to the continuous GF phase of the system. Proteins as large molecules were considered as dispersed particles within the carbohydrate phase. At maximum freeze-concentration, a "syrup" with 2.5 mol of water per mol of hexose unit is known to remain unfrozen in sugar solutions (Roos and Karel 1991a, b), while the whey protein isolate (WPI)–GF shows an increased 2.75 mol H_2O/mol GF, implying an increase in hydrogen bonding towards the protein hydration interface. At low temperatures and under lyophilization conditions, proteins with carbohydrate cryoprotectants exist with unfrozen hydration water exposed to either a solid (glassy) or liquid (rubbery) unfrozen aqueous carbohydrate phase. A liquid carbohydrate phase causes significant osmotic stress which is not present in the vitrified state of the carbohydrate. According to Roos and Potes (2015), hydrated protein particles within cryoprotectant solutions become encapsulated by the vitrifying carbohydrate phase. Such a

mechanism explains the retention of protein conformation in freezing and dehydration by freeze-drying. In other dehydration processes, glass formation around hydrated protein by a protectant carbohydrate can also explain the stabilization of protein conformation and structure.

9.6 The Freeze-Drying Process

Freeze-drying (lyophilization) involves the freezing of water to ice and establishment of drying conditions where sublimation of ice to water vapor is accelerated and the vapor is condensed as ice on a cold surface. Such conditions can be established by lowering the temperature or using vacuum equipment to lower the pressure of a drying chamber to control the temperature of the ice.

The freeze-drying process is described in Fig. 9.4. A typical process uses vacuum equipment to lower the pressure of the drying chamber to $p < 1$ mbar. At such conditions, the ice temperature, T_{ice}, is a direct function of the surrounding pressure. On the other hand, refrigeration equipment is used to lower the temperature of a condensing surface, T_c, to below the temperature of the sublimating ice. This allows for a condition where the vapor pressure of the ice, p_{ice}, of the drying material is higher than that found on the condenser surface, p_c. Hence, the driving force for sublimation is defined by the vapor pressure difference, $\Delta p = p_{ice} - p_c$. Obviously, an increase in Δp accelerates the freeze-drying process.

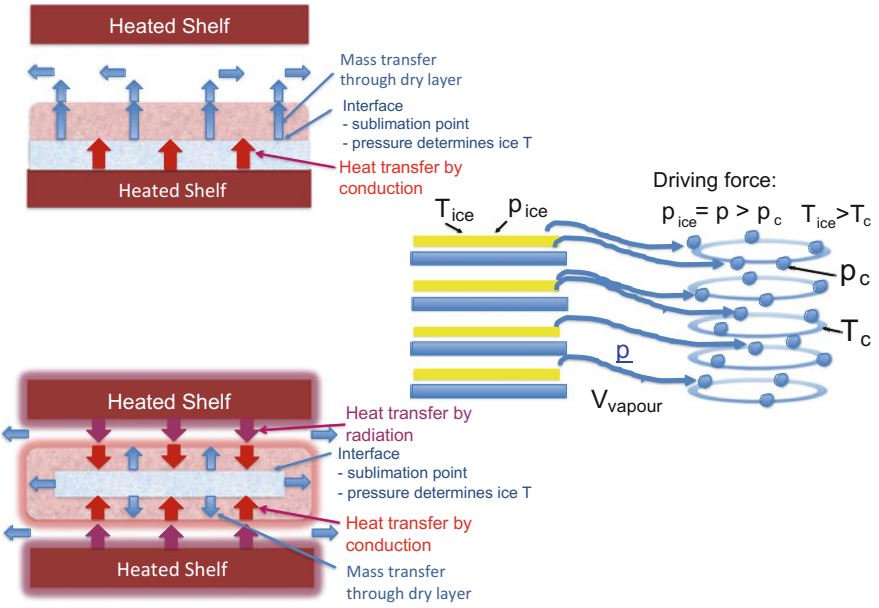

Fig. 9.4 The principle of freeze-drying (*right*) and heat transfer and mass transfer to accelerate and control freeze-drying when by conduction (*left top*) or radiation (*left bottom*)

Solutes in the water phase of biological materials affect the freezing of water, and they each have a specific temperature for maximum freeze-concentration and corresponding onset temperature of ice melting, T_m'. A prerequisite for freeze-drying is the solid state for the sublimating ice, and T_m' thus provides a critical temperature for sublimation in freeze-drying. Successful freeze-drying requires that the temperature for sublimation is lower than the T_m' of the water–solutes phase to avoid melting during sublimation. The ice temperature is affected by the chamber pressure and also by the heat and mass transfer within the material undergoing freeze-drying. Heat supplied to the sublimating ice can accelerate freeze-drying, while mass transport inside the drying particle controls the internal pressure of the drying particle (Fig. 9.4). This also causes large differences between freeze-drying processes using radiation and contact heat transfer. As described in Fig. 9.4, during freeze-drying, ice is an excellent heat conductor (heat transfer by conduction through an ice layer on a heated shelf), while an already dried porous layer of a biological material is an efficient insulator for heat transfer (radiation from heated plates and heat conduction to sublimation interface within a drying particle).

Conditions which must be satisfied during a successful freeze-drying process are shown in Fig. 9.5. A preferably external pre-freezing step of a material must ensure that the freezing step produces a desired size of ice crystals and surrounding external wall of unfrozen solids phase thickness (Harnkarnsujarit et al. 2012). Thereafter, the ice temperature must be lowered to well below the T_m', with corresponding ice vapor pressure of $p_{ice} < p_m'$, to ensure that $T_{ice} < T_m'$ applies during loading of the freeze-dryer until proper freeze-drying conditions for maintaining $T_{ice} < T_m'$ have been established. During any condition of $T_{ice} > T_m'$ corresponding to $p_{ice} > p_m'$, a liquid water phase appears and results in viscous flow within the unfrozen water–solutes phase. Such conditions lead to shrinkage and collapse of the structure of the drying material, and often to poor dehydration and loss of quality.

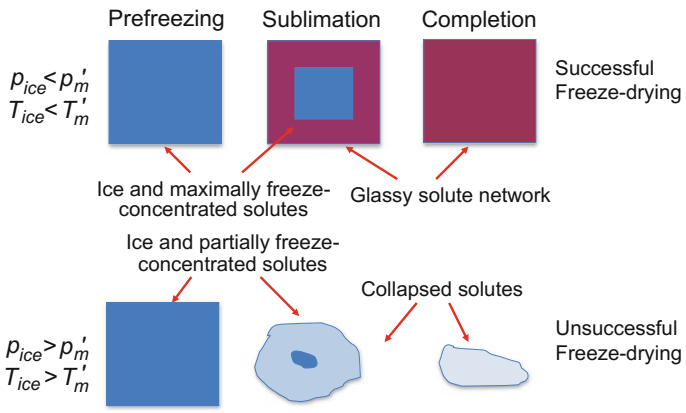

Fig. 9.5 Control of a freeze-drying process using information on ice melting properties to maintain ice sublimation at temperatures below the onset temperature of ice melting, T_m'

9.7 Freezing and Freeze-Drying of Microbes

The survival of starter cultures and preservation of bioactive micro-organisms, particularly lactic acid bacteria, are of fundamental importance in preserving viable probiotic cultures for food use, as well as in the development of dehydrated foods with probiotic activity. Frozen culture concentrates of probiotics exhibit maximal survival in liquid nitrogen (Smittle et al. 1974), while freeze-drying is one of the most successful and convenient methods of preserving microbial cultures (Berny and Hennebert 1991; Carvalho et al. 2003).

The major loss of cell viability due to freezing is attributed mainly to poor freezing or freeze-drying conditions that affect ice crystal formation, high osmotic stress due to high concentrations of internal solutes of cells, leading to membrane damage, and macromolecular denaturation and the removal of water, which affect the properties of many hydrophilic biological macromolecules (Thammavongs et al. 1996). Franks et al. (1991) showed that the activity of proteins was substantially reduced during freeze-drying, and was further reduced by subsequent storage unless the dried material was kept below −20 °C, and ideally at −80 °C. Such finding suggested that stability of protein materials may be achieved at conditions supporting vitrification of protein hydration water and associated low mobility of macromolecular components (Roos and Potes 2015).

Cryoprotectant molecules used in freezing and freeze-drying are often small sugars such as glucose and sucrose, while lactose may be also present depending on the fermentation media. Skim milk and/or combinations of sucrose, trehalose and dextran are most commonly used as cryoprotectants (Hubalek 2003). Sugars exhibit typical glass-forming properties of carbohydrates, and they have a significant impact on freezing, freeze-drying and storage characteristics. Such properties were reported for common sugars by Roos (1993), and are useful in the selection of cryoprotectant systems and freezing and freeze-drying conditions. Reconstituted skim milk solids are among the most useful suspension media for the freezing or freeze-drying of starter cultures due to their cryoprotective effects on cells (Abraham et al. 1990), and supplementing skim milk solids with different cryoprotectants may increase the cryoprotective effect (King and Lin 1995). A large number of compounds have been assessed for use as cryoprotectants, including skim milk solids, glycerol, mannitol, sorbitol, trehalose, sucrose, maltose, lactose, fructose, glucose, betaine, monosodium glutamate, honey, and amino acids and their salts.

A typical protectant should provide cryoprotection of probiotic bacteria cells during freezing and freeze-drying processes and ease of rehydration after long-term storage (Costa et al. 2000). Protectants present in a suspending medium have two main modes of action in preserving the viability of freeze-dried cells. Firstly, a glass-forming carbohydrate will provide mechanical support during storage. Secondly, the living cells are biochemically protected against damage during the drying process and subsequent storage (Berny and Hennebert 1991). Stabilization by small carbohydrate molecules has also been explained using the "water replacement hypothesis", which refers to their capacity to form hydrogen bonds

with components of biological structures in the absence of water (Crowe et al. 2001). The ability of skim milk to protect freeze-dried microorganisms is thought to be due to its capacity to stabilize the cell membrane constituents and to create a porous structure in the freeze-dried materials (Selmer-Olsen et al. 1999).

Interestingly, sugar composition within microbial cells cannot be assumed to be the same as that of a cryoprotectant medium. Sugar metabolism involves sugar uptake into the cell and breakdown into simpler products that are easily metabolized for energy. In lactic acid bacteria, there are two systems for the transport and metabolism of lactose. The two methods for transporting lactose into the cells are as follows (Walstra et al. 1999):

1. A phosphoenol pyruvate-dependent phosphotransferase system (PEP/PTS): As lactose is transported into the cell, it is transformed to lactose-P. Once inside the cell, phospho-β-galactosidase (P-β-gal) hydrolyzes the lactose-P to glucose and galactose-6-P. Glucose is further converted to glucose-P, and both phosphated sugars are further metabolized.
2. An ATP-dependent permease system: Lactose is transported into the cell as described above, and hydrolyzed by β-galactosidase or lactase into glucose and galactose. Glucose is converted to glucose 6-P. In galactose-fermenting bacteria, galactose is converted to glucose-6-P by the Leloir pathway.

Galactose is not metabolized if enzymes of the Leloir pathway are not present (i.e., *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*). Therefore, galactose present in such probiotic cells is either secreted or converted to (exo) polysaccharides (Walstra et al. 1999). Obviously, fermentation conditions and cell composition at the time of freezing may have an important effect on cell viability in both freezing and freeze-drying, as intracellular sugar composition may show large variations. Fonseca et al. (2000) found that the larger the surface area of the cell, the greater the membrane damage, owing to extracellular ice crystal formation during freezing. Water content as such is also an important factor affecting the viability of bacteria during freeze-drying and subsequent storage (Zayed and Roos 2004).

Crowe et al. (1984) reported that the role of trehalose in the stabilization of dry biological membranes was by hydrogen bonding to the polar head group of the phospholipid membrane. In addition to possible hydrogen bonding to cell membranes, trehalose and sucrose as glass formers play a role in protecting cell membranes from fusion and breakage (Crowe et al. 1984). Such carbohydrates also stabilize proteins against denaturation during freezing and freeze-drying (Crowe et al. 1988; Franks et al. 1991). Zayed and Roos (2004) demonstrated that the capacity of lactic acid bacteria (LAB) to metabolize trehalose did not seem to confer greater resistance following use as a protectant during the freeze-drying process. Pehkonen et al. (2008) also showed that maximum ice formation during pre-freezing to satisfy the condition $T_{ice} < T_m'$ was more important than the use of lactose, trehalose or their mixture as cryoprotectant for *Lactobacillus rhamnosus* GG. Post-freeze-drying conditions require maintenance of the glassy state of cryoprotectants and possible cell constituents which typically require storage in hermetic packages in the absence of water, at almost "zero" water content

and relative humidity of approximately 0 % (Pehkonen et al. 2008). Pehkonen et al. (2008) reported slightly improved stability for *L. rhamnosus* GG when freeze-dried with trehalose or lactose–trehalose compared to lactose. Such differences in storage stability with various cryoprotectants may depend primarily on the intracellular sugar composition, which may be strongly affected by cell activity in the cryoprotectant system under pre-freezing conditions (Pehkonen et al. 2008).

9.8 Intracellular Sugar Composition

Although there is limited knowledge regarding the intracellular sugar composition of living cells during fermentation and freezing, we analyzed sugar utilization by selected probiotic bacteria to evaluate the effects of cryoprotectants on their intracellular sugar composition for freezing and freeze-drying applications. *L. acidophilus* was able to ferment several sugars, while *L. casei* fermented lactose, trehalose, glucose and galactose. *L. bulgaricus* grew successfully in maltose, lactose and glucose. *Lactobacillus viridescens* showed positive results for maltose and glucose, whereas *Lactobacillus sakei* gave positive results for such sugars except lactose (Table 9.1).

We further studied the sugar composition of *Lactobacillus acidophilus* and *Lactobacillus casei* cells grown with skim milk and various sugars containing growth media. Probiotic cultures were washed and sonicated to remove extracellular sugars and release cell contents, respectively. Sonicated *L. acidophilus* cells showed little or no maltose uptake, and glucose concentrations remained low. These data showed that upon entering the cell, maltose was immediately hydrolyzed into two glucose moieties and utilized by *L. acidophilus*. A significant accumulation of maltose within cells was found for *L. casei*.

Galactose, glucose and lactose content in both *L. acidophilus* and *L. casei* varied depending on the length of fermentation, as shown in Fig. 9.6. Lactose was readily accumulated over time in the cells of both strains. Lactose uptake more than quadrupled from 5 min to 1 h after inoculation of *L. casei* into 15 % (w/w) lactose solution, and the intracellular lactose concentration reached 10 g/100 g of solids at

Table 9.1 Sugars fermented by *Lactobacillus* species

Strains	*L. acidophilus*	*L. casei*	*L. bulgaricus*	*L. sakei*	*L. viridescens*
Sugars					
Sucrose	+	−	−	+	−
Lactose	+	+	+	−	−
Maltose	+	−	+	+	+
Trehalose	+	+	−	+	−
Glucose	+	+	+	+	+
Galactose	+	+	−	+	−

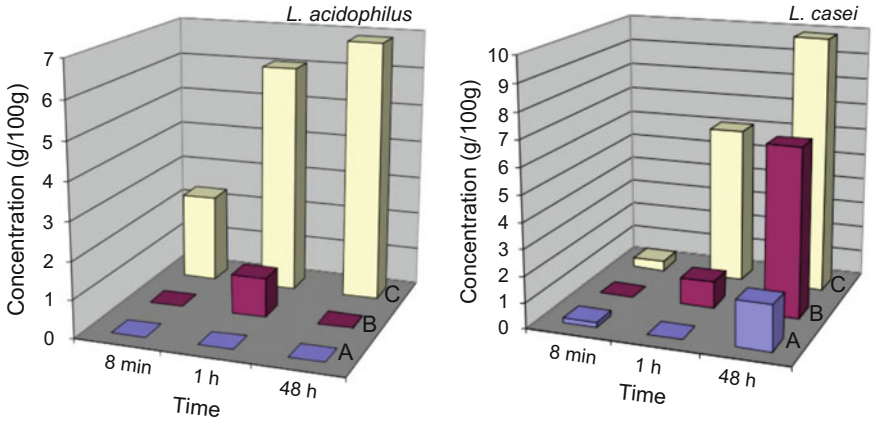

Fig. 9.6 Intracellular galactose (*A*), glucose (*B*) and lactose (*C*) content in *L. acidophilus* and *L. casei* cells during growth in 15 % (w/w) lactose solution

48 h. In addition to the high uptake of lactose, its hydrolysis to glucose and galactose in *L. casei* cells was confirmed by high concentrations of intracellular monosaccharides, especially glucose. The glucose concentration increased steadily with time to a little over 6/100 g of solids, and the galactose reached a concentration of 1.7/100 g of solids at 48 h (Fig. 9.6).

Lactose uptake by the *L. acidophilus* cells was similar to that of the *L. casei* cells, but the accumulated concentration was not as high, with a maximum concentration of only 6.8/100 g of solution accumulating after 48 h. The concentration of glucose within the *L. acidophilus* varied.

Our study showed greater disaccharide utilization by *L. acidophilus* than by *L. casei* while variations in intracellular composition implied significant differences in intracellular freezing and freeze-drying properties. Sonication of the cells at different time points showed that lactose was accumulated almost immediately by *L. acidophilus*, and that its concentration was proportional to the length of time of immersion in the media. However, *L. casei* showed uptake of a larger concentration of lactose after 48 h than *L. acidophilus*, and also showed a much greater increase in monosaccharide formation. The other disaccharides showed a similar pattern, with the exception of maltose. The maltose concentration within the cells was quite low and remained constant up to 48 h; however, accumulation within the *L. casei* cells after 7 days suggested that maltose uptake was directly proportional to utilization needs, and only accumulated over time in a saturated environment.

9.9 Conclusions

Freezing and freeze-drying are important technologies for the stabilization of bioactive materials used in the food industry as ingredients or by consumers in foods and supplements. The effects of freezing have been studied in biological materials, but little information is available on the properties of water and ice in complex biological materials at low temperatures. A better understanding of complex carbohydrate–protein–lipid systems in freezing and freeze-drying is needed in order to improve the stability of bioactive materials during freezing, thawing, drying and reconstitution.

References

Abraham A, De Antoni GL, Anon MC (1990) Microbiology of starter cultures. Cryobiology 27:336–342

Angell CA (2002) Liquid fragility and the glass transition in water and aqueous solutions. Chem Rev 102:2627–2650

Angell CA (2008) Insights into phases of liquid water from study of its unusual glass-forming properties. Science 319:582–587

Angell CA (2014) Supercooled water. Two phases? Nat Mater 13:673–675

Atkins P, De Paula J (2010) Atkin's physical chemistry, 9th edn, 972 p. Oxford University Press, Oxford

Berny JK, Hennebert GL (1991) Viability and stability of yeast cells and filamentous fungus spores during freeze-drying: effects of protectants and cooling rates. Mycologia 83:805–815

Buera MP, Roos YH, Levine H, Slade L, Corti HR, Auffret T, Angell CA (2011) State diagrams for improving processing and storage of foods, biological materials and pharmaceuticals. Pure Appl Chem 83:1567–1617

Carvalho AS, Silva J, Ho P, Teixeira P, Malcata FX, Gibbs P (2003) Protective effect of sorbitol and monosodium glutamate during storage of freeze-dried lactic acid bacteria. Lait 83:203–210

Corti HR, Angell CA, Auffret T, Levine H, Buera MP, Reid DS, Roos YH, Slade L (2010) Empirical and theoretical models of equilibrium and non-equilibrium transition temperatures of supplemented phase diagrams in aqueous systems. Pure Appl Chem 82(5):1065–1097

Costa E, Usall J, Teixido N, Garcia N, Vinas I (2000) Effect of protective agents, rehydration media and initial cell concentration on viability of *Pantoea agglomerans* strain CPA-2 subjected to freeze-drying. J Appl Microbiol 89:793–800

Crowe JH, Crowe LM, Chapman D (1984) Preservation of membranes in anhydrobiotic organisms: the role of trehalose. Science 223:701–703

Crowe JH, Crowe LM, Carpenter JK, Rudolph AS, Winstrom CA, Spargo BJ, Anchordoguy TJ (1988) Interactions of sugars and membranes. Biochim Biophys Acta 947:367–384

Crowe JH, Crowe LM, Oliver AE, Tsvetkova N, Wolkers W, Tablin F (2001) The trehalose myth revisited: introduction to a symposium on stabilization of cells in the dry state. Cryobiology 43:89–105

Doster W, Bachleitner A, Dunau R, Hiebl M, Lüscher E (1986) Thermal properties of water in myoglobin crystals and solutions at subzero temperatures. Biophys J 50:213–219

Fonseca F, Beal C, Corrieu G (2000) Method for quantifying the loss of acidification activity of lactic acid starters during freezing and frozen storage. J Dairy Res 67:83–90

Franks F, Asquith MH, Hammond CC, Skaer HB, Echlin P (1977) Polymeric cryoprotectants in the preservation of biological ultrastructure I. Low temperature states of aqueous solutions of hydrophilic polymers. J Microsc (Oxford) 110:223–238

Franks F, Hatley RHM, Mathias SF (1991) Material science and the production of shelf-stable biologicals. Biopharmaceuticals 4:38–42

Harnkarnsujarit N, Charoenrein S, Roos YH (2012) Microstructure formation of maltodextrin and sugar matrices in freeze-dried systems. Carbohydr Polym 88:734–742

Hubalek Z (2003) Protectants used in the cryopreservation of microorganisms. Cryobiology 46:205–229

Jansson H, Bergman R, Swenson J (2011) Role of solvent for the dynamics and the glass transition in proteins. J Phys Chem B 115:4099–4109

Jorgensen L, Hostrupb S, Horn Moeller E, Grohganz H (2009) Recent trends in stabilising peptides and proteins in pharmaceutical formulation—considerations in the choice of excipients. Expert Opin Drug Deliv 6:1219–1230

King VAE, Lin HJ (1995) Studies on the effect of protectants on *Lactobacillus acidophilus* strain dehydrated under controlled low-temperature vacuum dehydration and freeze-drying by using response surface methodology. J Sci Food Agric 68:191–196

Levine H, Slade L (1986) A polymer physico-chemical approach to the study of commercial starch hydrolysis products (SHPs). Carbohydr Polym 6:213–244

Luyet B, Rasmussen D (1968) Study by differential thermal analysis of the temperatures of instability of rapidly cooled solutions of glycerol, ethylene glycol, sucrose and glucose. Biodynamica 10(211):167–191

Pehkonen KS, Roos YH, Song M, Ross RP, Stanton C (2008) State transitions and physicochemical aspects of cryoprotection and stabilization in freeze-drying of *Lactobacillus rhamnosus* GG (LGG). J Appl Microbiol 104:1732–1743

Roos Y (1993) Melting and glass transitions of low molecular weight carbohydrates. Carbohydr Res 238:39–48

Roos YH, Drusch S (2015) Phase transitions in foods, 2nd edn 366 p. Academic Press, Inc., San Diego

Roos YH, Karel M (1991a) Applying state diagrams to food processing and development. Food Technol 45(66):68–71, 107

Roos YH, Karel M (1991b) Amorphous state and delayed ice formation in sucrose solutions. Int J Food Sci Technol 26:553–566

Roos YH, Potes N (2015) Quantification of protein hydration, glass transitions and structural relaxations of aqueous protein and carbohydrate-protein systems. J Phys Chem B 119:7077–7086

Selmer-Olsen E, Birkeland SE, Sorhaug T (1999) Effect of protective solutes on leakage from and survival of immobilized *Lactobacillus* subjected to drying, storage and rehydration. J Appl Microbiol 87:429–437

Smittle RB, Gilland SE, Speck ML, Walter Jr (1974) Relationship of cellular fatty acid composition to survival of *Lactobacillus bulgaricus* in liquid nitrogen. J Appl Microbiol 27:738–741

Swenson J, Cerveny S (2015) Dynamics of deeply supercooled interfacial water. J Phys: Condens Matter 27:033102

Thammavongs B, Corroler D, Panoff JM, Auffray Y, Boutibonnes P (1996) Physiological response of *Entercoccus faecalis* JH2-2 to cold shock: growth at low temperatures and freezing/thawing challenge. Lett Appl Microbiol 23:398–402

Walstra P, Geurts TJ, Noomen A, Jellema A, van Boekel MAJS (1999) Dairy technology: priniciples of milk properties and processes. Marcel Dekker Inc., New York, pp 325–362

Zayed G, Roos YH (2004) Influence of trehalose and moisture content on survival of *Lactobacillus salivarius* subjected to freeze-drying and storage. Process Biochem 39:1081–1086

Chapter 10
Spray Drying of Bioactives

Zhongxiang Fang and Bhesh Bhandari

Abstract Spray drying is a common unit operation for converting solids from liquid materials into powders for preservation, ease of storage, transport and handling, and economic considerations. Although most often considered as a dehydration process, spray drying can also be effective as an encapsulation method when it is used for complexing a core material with a protective matrix, which is ideally inert to the core material being encapsulated. Unlike other encapsulation techniques, it offers the unique advantage of producing microcapsules in a cost-effective one-step continuous process. This chapter describes the principles and processing techniques of spray drying for encapsulation of food bioactives, including probiotics, polyphenols, enzymes and peptides, vitamins, and essential fatty acids. The storage stability of spray dried bioactives and challenges from both a research and industrial perspective are also briefly discussed.

Keywords Spray drying · Encapsulation · Bioactive compounds · Stability

10.1 Introduction

Over the past decade, there has been an increasing awareness regarding the importance of maintaining overall health and wellness by adopting a positive lifestyle and consuming a healthy diet. Health-promoting foods are those considered to be beneficial to health in ways that go beyond a normal healthy diet required for human nutrition, and the term may also refer to functional foods that are designed to address specific health concerns or disease prevention (Roberfroid

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2000). Food components that contribute to these health benefits are bioactive compounds typically including vitamins, ω-3 fatty acids, peptides, and phytochemicals. However, most bioactive compounds are extra-nutritional constituents that occur only in small quantities in foods (Kris-Etherton et al. 2002), and their stability and functionality are highly dependent on processing and storage conditions. Research on the extraction and isolation of bioactives and on the development of bioactive supplements for human health is a hot topic in both academic and industrial communities (Vinatoru 2001; Kitts and Weiler 2003; Puri et al. 2012).

One practical means of preserving stability and bioactivity of these bioactive compounds is through the selection of a suitable processing method and storage of the compounds in a dry glassy state to reduce deterioration (Ludescher et al. 2001). Spray drying is a well-established method for transforming solids from liquid materials into solid dry powders. With the advantages of economical, flexible, continuous operation, and resulting powder with good flowability, this technique has been widely applied in chemical, food, biochemical, and pharmaceutical industries (Broadhead et al. 1992; Bhandari et al. 2008). Because the drying time is very short (generally 15–30 s) for the passage of the sprayed particle, and intense evaporation occurs at the surface to maintain the droplet at an almost cool temperature until a dry state is reached (Fogler and Kleinschmidt 1938; Gharsallaoui et al. 2007), spray drying is commonly used for drying of heat-sensitive materials containing flavors, enzymes, and probiotics (Ré 1998; Jafari et al. 2008b). In addition, as the spray dried material has a matrix structure that can entrap active (core) materials, spray drying is also used to encapsulate food ingredients (Gharsallaoui et al. 2007) and food bioactives (Fang and Bhandari 2010), which impart numerous benefits to the encapsulated compounds including significantly improved stability, bioactivity, and targeted release (Augustin and Hemar 2009; de Vos et al. 2010). For example, spray drying of milk can be considered as an encapsulation process where milk fat is the core material that is protected against oxidation by a wall material consisting of a mix of lactose and milk proteins (Gharsallaoui et al. 2007). It is therefore understandable that an encapsulation effect is desirable for the spray drying of bioactive compounds. This chapter will provide an overview of the spray drying of food solids with bioactives, and the stability of the powders will be discussed.

10.2 The Spray Drying Process

The concept of spray drying was first reported in 1872 in a patent by Percy (1872). The technique was introduced for commercial purposes in 1903, and was fully established on a large-scale basis in the early 1950s (Masters 1991; Bhandari et al. 2008). The basic process of spray drying is illustrated in Fig. 10.1. Briefly, it involves feeding a prepared liquid into a spray dryer, and atomization with a nozzle or rotating disc in a chamber supplied with dry hot air. The droplet comes into contact with the hot air in the chamber, causing evaporation of the solvent, and the dried particles are then separated and the fines removed from the humid air using a cyclone or bag filter (Ré

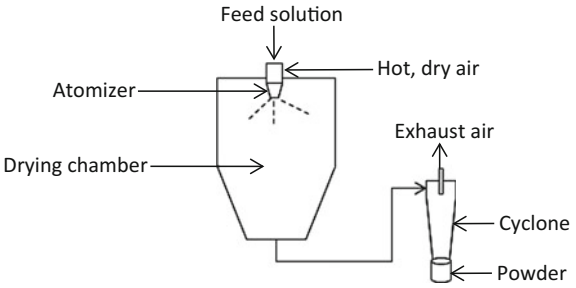

Fig. 10.1 Schematic of typical spray drying process

1998; Gibbs, et al. 1999; Zuidam and Shimoni 2010; Fang and Bhandari 2012a). Although less common, spray drying of ingredients from an organic solution such as acetone or ethanol may be performed if the materials are not water-soluble and are very heat-sensitive, requiring low-temperature drying. The spray drying process used for encapsulation of food bioactives is similar to that of dehydration, except that the feed solution preparation step may involve dissolving, emulsifying, or dispersing the core material in an aqueous medium with the selected wall material. The spray dried particles generally form a spherical matrix structure, but particle size may vary from very fine (10–50 μm) to large (2–3 mm) if spray drying is combined with an agglomeration process (Gharsallaoui et al. 2007). Particle sizes ranging from 300 nm to 5 μm were recently achieved with a BÜCHI Nano Spray Dryer B-90, which uses a vibrating mesh technology to generate fine droplets (Schmid et al. 2011).

10.2.1 Preparation of Feed Solution

Methods for preparing feed solutions are dependent on the nature of the material being spray dried and the end use of the powder. Water-soluble materials can be dissolved in water, but an oil-in-water emulsion should be prepared for oils or oil-soluble materials, and a solid/liquid suspension can be formed by dispersing a solid or particulate material (e.g. probiotics) in a solvent (Fang and Bhandari 2012a). It should be noted that if spray drying materials are sugar-rich products such as fruit/vegetable juice or honey, high molecular weight additives (starches, maltodextrins) or surfactants (proteins) may be added to overcome the problems of stickiness and agglomeration in the drying chamber (Bhandari et al. 2008; Fang and Bhandari 2012b; Fang et al. 2013;). For encapsulation, a wall material is selected and incorporated into the feed solution/emulsion/dispersion to protect the core materials. The wall material should have specific properties, including good film-forming and emulsifying ability, high solubility in water, low viscosity with high solids levels, low hygroscopicity, protection and controllable release of the core material, a stable and low-cost supply, and bland taste (Ré 1998; Desai and Park 2005; Augustin and Hemar 2009). Examples of two commonly used wall materials, carbohydrates and proteins, are presented in Table 10.1, along with the properties that they provide (Fang and Bhandari 2012a).

Table 10.1 Some commonly used wall materials and their properties for spray drying encapsulation (Fang and Bhandari 2012a, with permission)

Wall material	Example	Encapsulation-related properties
Carbohydrates	Hydrolyzed starches: corn syrup solids, maltodextrins	Very good oxygen barrier; low viscosity at high solids; no/limited emulsion stabilization; low cost
	Modified starches: acetylated starch, monostarch phosphate	Good emulsion stabilization; varying quality; constricted usage due to regulatory situation; low cost
	Cyclodextrins: α-, β-, γ-cyclodextrins	Good inclusion of volatiles; excellent oxygen barrier; relatively expensive
	Gums: agar, acacia (arabic), xanthan, alginates	Good emulsions; very good retention of volatiles; varying quality; price depends on availability; sometimes impurities
Proteins	Milk proteins: whey proteins, caseinates, skim milk powders	Good emulsions; properties dependent on other factors such as pH and ionic strength; allergenic potential; relatively expensive
	Other proteins: soy protein, egg protein, gelatin	
Other biopolymers	Soluble soy polysaccharides, chitosan, Maillard reaction products, modified celluloses	Varied properties; may provide additional benefit to the stability of actives

10.2.2 Atomization

Atomization is one of the most important processing steps during spray drying. The goal of this stage is to create a maximum heat transfer surface area between the liquid droplets and the dry medium in order to optimize heat and mass transfer. Liquid atomization in small droplets can be generated by atomizers. Commonly used types include rotary wheel/disc (using centrifugal energy), pressure nozzles (using pressure energy), dual-fluid-spray nozzles (using pressure and gas energy), and sonic nozzles (using sonic energy) (Masters 1991; Bhandari et al. 2008). Atomizers can be used either as an individual device or in groups, but must be able to work effectively and reliably under harsh conditions. The selection and operation of the atomizer is critical in spray drying, because drying efficiency, particle size, size distribution, and powder collection efficiency are all dependent on atomizer performance. The choice of atomizer also depends on the nature and viscosity of the feed solution and the desired characteristics of the dried powder. For example, the use of a pressure nozzle will enable the formation of finer droplets with higher energy input. At a constant energy level, the size of the formed particles increases with increasing feed rate. However, particle size increases when both the viscosity and surface tension of the initial liquid are high (Gharsallaoui et al. 2007; Bhandari et al. 2008). Special consideration for atomizer selection is required in spray drying encapsulation, especially when the feed solutions are solid-in-oil-in-water (s/o/w) emulsions or emulsions with high viscosity, because blockage of the atomizer can

occur with solid core material due to large particle size or viscosity that is too high (Fang and Bhandari 2012a). It is important, therefore, to control the solid core particle size and viscosity of the liquid emulsion before spray drying and to select an appropriate atomizer for a specific feed solution.

10.2.3 Drying Process

The drying process is initiated upon contact of the atomized liquid droplets with hot air in the drying chamber. Based on atomizer emplacement in relation to the inlet of hot air, spray drying can be classified as co-current or counter-current drying. In co-current drying, the hot air and the atomized droplets move in the same direction in the drying chamber, whereas with a counter-current drying design, the flow of hot air and the droplets move in opposite directions. During a co-current drying process, the mean residence time of the particles is small, and the dried particles do not have to pass through the high-temperature zone; therefore, it is suitable for drying thermo-sensitive materials such as bioactive compounds (Bhandari et al. 2008). Typically, the hot air inlet temperature ranges from 150 to 220 °C, and evaporation occurs instantaneously. The dry powders are thus exposed to moderate temperatures ($\sim$50–80 °C), which limits their thermal degradation (Fleming 1921; Gharsallaoui et al. 2007). For spray drying encapsulation, the short time exposure to keep the core temperature below 40 °C helps to prevent damage to the product (Dubernet and Benoit 1986). On the other hand, counter-current spray drying also has advantages. Because the dry product will be exposed to high temperatures during the drying process, the powders will generally have low final moisture content, which is of benefit to the shelf life of the dried product. Another advantage of the counter-current process is that it is considered more economical in terms of energy savings (Gharsallaoui et al. 2007).

At the time of droplet–hot air contact, heat transfer from the air towards the product occurs as a result of the temperature difference, and the transfer of water is carried out in the opposite direction due to the vapor pressure difference. Therefore, a balance of temperature and vapor partial pressure is established between the liquid and gas phases, and the loss of moisture from the droplet is controlled by the gas phase resistance. This is called the constant-rate or constant-activity period, as the water activity at the droplet surface remains nearly constant (Ré 1998). If the partial pressure of water vapor in the bulk air has not built up to a substantial value, the water vapor partial pressure driving force for mass transfer in the gas boundary layer surrounding the droplet remains constant, and the evaporation rate per unit area of droplet surface is constant. As heat continues to transfer by convection from the drying air to the surface of the droplet, water continues to evaporate from the surface. The water is lost in proportion to the heat gained, and the droplet temperature is the wet-bulb temperature of the drying air. As drying continues, a water concentration gradient is built up within the droplet, the water activity at the surface decreases, and the surface dries out. This brings about the falling-rate period, where drying is rate-limited by moisture transport within droplets. The relative lengths of the constant-rate and the falling-rate periods vary according to the conditions in the spray dryer and the material being dried. Once a

dry skin has been formed, the droplet temperature starts to increase from the wet-bulb to the air temperature. At temperatures reaching or exceeding the boiling point of water, substantial internal voids and particle inflation tend to occur. When the particle is sufficiently dry, the final shape sets into place, and any final evaporation of water occurs, thus completing the transformation of droplets into dry particles.

10.2.4 Recovery of the Spray Dried Powder

The dried powders are delivered from the drying chamber to a powder separator by the drying air flow. For modern multi-stage spray dryers, the air flow from the drying chamber typically contains about 10–50 % of the total powder, depending on the material and spray dryer type and operating conditions (Pisecky 1997). It is important to recover the powder from the exhaust flow not only for cost purposes, but also to clean the air in order to minimize pollution. In general, particles are recovered by incorporating one or more of the cyclone separators, bag filters, or wet scrubbers (Bhandari et al. 2008). With the use of a cyclone, particles hit the cyclone wall due to the centrifugal force and then drop down due to gravity, while the clean air moves up from the bottom to the top of the cyclone as a result of differential pressures created between the radial and axial directions. Bag filters are used in particular for the removal of the finest powders. With the wet scrubber, the exhaust air is injected at very high velocities through a venturi inlet. At the same time, a solvent fluid (normally water) is sprayed in such a way that the gas stream and water spray make intimate contact. The fine particles are dissolved in the water, and the clean air then escapes from the scrubber.

After separation, the obtained powder is composed of particles originating from spherical droplets with some degree of shrinkage. The particles can be compact or hollow, depending on the composition, drying temperature and water and gas content of the droplet (Bimbenet et al. 2002). A fluidized bed is sometimes integrated into the spray dryer to reduce drying cost, better control the particle size, or produce powders with very low water content (Fang and Bhandari 2012a). In addition, an agglomeration process may be utilized after spray drying to improve the rehydration ability of the dried particles.

10.3 Applications of Spray Drying for Drying of Bioactives

Bioactive compounds *can be defined as natural essential and non-essential compounds (e.g. vitamins or polyphenols) in the food chain that have an effect on human health* (Biesalski et al. 2009). *Because of their sensitivity to adverse environments such as high temperature, light, and oxygen, it is important to select a suitable processing method to preserve their stability, and therefore their bioactivity. Spray drying is a widely accepted and economical method for drying of heat-sensitive materials* (Bhandari et al. 2008)*, including bioactive compounds.*

10.3.1 Probiotics

The word 'probiotics' is derived from the Latin 'pro' and the Greek 'biotic', meaning 'for life', and was defined by Fuller (1992) *as 'a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance'*. Probiotics can survive gastric, bile, and pancreatic secretions, attach to epithelial cells, and colonize the human intestine. Microbes from many different genera are currently used as probiotics, among which the most common strains are *Lactobacillus* spp. (*L. acidophilus, L. casei, L. johnsonii, L. plantarum*), *Bifidobacterium* spp. (*B. bifidum, B. animalis, B. breve*), and yeasts (*Saccharomyces boulardii*) (Alvarez-Olmos and Oberhelman 2001). Probiotics have been reported to provide a variety of beneficial health effects and are used in a number of therapeutic applications, including maintenance of normal gastrointestinal tract microflora, improvement of constipation, treatment of diarrhea, enhancement of the immune system, alleviation of lactose intolerance, reduction of allergy risk in infancy, reduction of serum cholesterol levels, anticarcinogenic activity, and improved nutritional value of foods (Kailasapathy and Chin 2000; Mattila-Sandholm et al. 2002; Touhy et al. 2003). In the food industry, a large number of probiotic products are manufactured in the form of milk, drinking and frozen yogurts, probiotic cheeses, ice creams, dairy spreads, and fermented soy products (Manojlović et al. 2010).

For spray drying of probiotics, the low survival rate of the microorganism during drying and low stability upon storage are the major factors limiting its commercialization (Manojlović et al. 2010). The damage to the cell wall, cytoplasmic membrane, ribosome, and DNA during the heating process may be the main reasons for the loss of viability (Abee and Wouters 1999). As discussed by Peighambardoust et al. (2011), the viability of probiotics during spray drying is dependent on a wide range of factors, including biological parameters (species, growth media, growth phase, intrinsic stress tolerance), processing parameters (inlet and outlet temperature, drying time, nozzle pressure), product parameters (carrier medium, concentration), pretreatments (stress response, protective substances), and post-drying conditions (rehydration, packaging, and storage).

The survival of probiotics during spray drying depends on the air temperature, types and strains of probiotics, and type of protective agent used. Moderate spray drying temperature conditions (air inlet temperature <160 °C) are usually selected for drying probiotics, as a very high temperature is detrimental to the viability of the probiotic bacteria (Anal and Singh 2007; Chavez and Ledeboer 2007). In one study, a lower inlet air temperature of 100 °C and outlet air temperature of 45 °C was found to obtain higher viability for spray drying encapsulation of *Bifidobacterium* sp. than conditions of higher inlet (>120 °C) and outlet (>60 °C) temperatures (O'Riordan et al. 2001). Other researchers have reported that the survival of probiotics declined with increasing inlet temperature (Mauriello et al. 1999; Gardiner et al. 2000), whereas outlet temperature was even more crucial to their survival (Ananta et al. 2005; Chavez and Ledeboer 2007; To and Etzel 1997). However,

care should be taken to ensure that the powder obtained at lower outlet temperatures has been dried sufficiently, because higher moisture content and higher water activity is obtained after low inlet/outlet temperature drying that will affect storage stability. The survival of probiotics is optimal at low water activity (<0.25) and low temperatures during storage, and a nitrogen- or vacuum-sealed package with a proper barrier function is recommended for storage of spray dried probiotics (Manojlović et al. 2010).

The viability of distinct species of a given genus and the growth phase differ under the same drying or storage conditions (Simpson et al. 2005). One group reported that the survival rate after spray drying was greatest for *Streptococcus thermophilus*, followed by *Lactobacillus paracasei* ssp. *paracasei*, with *L. lactis* ssp. *cremoris* found to be the least heat-tolerant microorganism (To and Etzel 1997). Lactic acid bacteria harvested at the stationary phase showed enhanced viability after spray drying (Corcoran et al. 2004), mainly due to the depletion of nutrients and glucose starvation in bacterial cells in the stationary growth phase. The cells demonstrated resistance to many environmental stresses, including osmotic and heat stress (Van de Guchte et al. 2002). Some studies have reported that the type of nozzle can influence the survival of the spray dried probiotics. An ultrasonic atomization nozzle provided smaller and more uniform particles compared to the traditional centrifugal and stationary dual-fluid spray nozzles, and resulted in smaller numbers of microorganism after drying (Al and Al 2009). The use of relatively low nozzle pressure has also been suggested to avoid high shear stress on the probiotics. For example, higher viability of *Lactobacillus acidophilus* was achieved after spray drying when the atomization pressure was reduced from 100 to 50 kPa (Riveros et al. 2009), and increased survival was reported for *Lactobacillus bulgaricus* when a spray pressure of 100 kPa instead of 200 kPa was used (Lievense and van Riet 1994).

The addition of prebiotics can also improve the survival of probiotics during spray drying and storage. It was reported that adding prebiotic oligosaccharides to whey protein isolate, pectin, and carboxymethylcellulose improved the survival of spray dried *L. acidophilus* (Vila-Garcia et al. 2010). Spray drying of the prebiotics inulin and oligofructose were reported to increase the storage survival rate of *Bifidobacterium* BB-12 (Fritzen-Freire et al. 2011). These prebiotics act as protective agents and sometimes also as carrier materials that reduce heat and shear stresses during drying and storage. Other protective agents include simple or complex components such as sugars (e.g. glucose, fructose, lactose, mannose, sucrose, sorbitol, adonitol, trehalose), ascorbic acid, skim milk, acacia gum, monosodium glutamate, and starch (Peighambardoust et al. 2011). Typically, these protective agents/carrier materials are combined with microorganisms at a concentration of about 10–20 % (w/w) (Lian et al. 2002; Ananta et al. 2005). Feed solutions with solid content that is too high (e.g. 40 %) produce larger particle sizes with longer drying times, and consequently greater thermal inactivation and less viability of the bacterial cells (Santivarangkna et al. 2007).

10.3.2 Polyphenols

Polyphenols are a group of plant secondary metabolites with a basic structure of several hydroxyl groups on aromatic rings (Manach et al. 2004). The most common and widely distributed group of polyphenols is flavonoids. Flavonoids are diphenylpropanes (C6-C3-C6) and consist of two aromatic rings linked through three carbons that usually form an oxygenated heterocycle (Fig. 10.2). Other common polyphenols include phenolic acids, stilbenes, and lignans, as presented in Fig. 10.2 (Manach et al. 2004). Interest in polyphenols has increased because of the recognition of their antioxidant properties, their great abundance in the human diet, and their likely role in the prevention of various diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases (Scalbert et al. 2005). In addition to antioxidant activity, polyphenols possess potential anti-inflammatory, antibacterial, and antiviral functions, suggesting a wide range of health benefits to humans (Surh 2003; Manach et al. 2004; Scalbert et al. 2005). However, most polyphenols are highly unstable under adverse environmental conditions such as unfavorable temperatures, light, pH, moisture, enzymes, and oxygen, and are therefore susceptible to degradative reactions in the course of food processing and storage or in the gastrointestinal tract (Bell 2001). Thus, the delivery of polyphenols requires protective properties that can maintain the active molecular form until the time of consumption, and that deliver this form to the physiological target within the organism. One natural characteristic of polyphenols is that they may have an unpleasant taste, such as astringency and bitterness, which needs to be masked before they are used in food or nutraceutical products (Haslam and Lilley 1988).

Spray drying has been used successfully for encapsulation of polyphenols and for maintaining their stability and antioxidant activity, as a number of studies have reported high polyphenol/antioxidant activity recovery (Table 10.2). Saéna et al. (2009) and Robert et al. (2010) found extremely high polyphenol recovery, exceeding 100 %, which may have been due to the hydrolysis of polyphenol conjugates during the preparation of the samples or during the drying process.

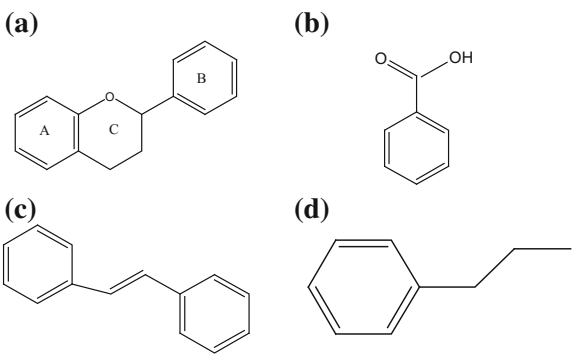

Fig. 10.2 Basic structures of polyphenols: **a** flavonoids, **b** phenolic acids, **c** stilbenes, and **d** lignans

However, high polyphenol degradation (65–68 %) was also reported by Georgetti et al. (2008), although the reasons were not mentioned, and the definition and calculation of polyphenol degradation in the study was unclear. Because most polyphenols are water-soluble compounds, an acceptable level of water solubility must also be present in the wall materials used in spray drying encapsulation, such as starch, maltodextrin, gum arabic, sodium caseinate, whey proteins, or their combinations (Table 10.2). The spray dried particles generally form a matrix structure of wall and core materials and have a spherical morphology.

In spray drying encapsulation of polyphenols, the air inlet temperature plays a critical role in polyphenol degradation; generally, the higher the temperature, the lower the polyphenol stability. In a study investigating spray drying of soybean extracts, an increase in the inlet gas temperature led to a product with a lower polyphenol concentration (Georgetti et al. 2008). In addition, greater loss of anthocyanin was observed with a higher (>180 °C) versus lower (160 °C) air inlet temperature (Ersus and Yurdagel 2007). The drying temperature is also affected by the encapsulation wall materials. For example, for spray drying encapsulation of cactus pear pulp and ethanol extract, an inlet temperature of 140 °C was used when

Table 10.2 Recent reports on spray drying encapsulation of polyphenols

Core materials (polyphenols)	Wall materials	Recovery	References
Bayberry juice (phenolic acids and flavonoids)	Maltodextrin and whey protein isolate	Polyphenols > 94 %	Fang and Bhandari (2011, 2012b)
Black carrot extracts (anthocyanins)	Maltodextrins	Not provided	Ersus and Yurdagel (2007)
Cactus pear pulp and extract (flavonoids and betalains)	Maltodextrin and inulin	Polyphenols > 100 %, betalains 62–100 %	Saéna et al. (2009)
Grape seed extract, apple polyphenol extract and olive leaf extract	Sodium caseinate-soy lecithin	Antioxidant activity 60–80 %	Kosaraju et al. (2008)
Hibiscus sabdariffa L. extract (anthocyanins)	Citrus fruit fiber	Not provided	Chiou and Langrish (2007)
Olive leaf extract	Chitosan	"No inactivation" of polyphenols	Kosaraju et al. (2006)
Pomegranate juice and ethanol extract (anthocyanins)	Maltodextrin and soybean protein isolates	96–121 %	Robert et al. (2010)
Procyanidins	Maltodextrin and gum arabic	Procyanidins > 89 %	Zhang et al. (2007)
Soybean extract	Colloidal silicon dioxide, maltodextrin, and starch	32–36 %	Georgetti et al. (2008)

maltodextrin was used as wall material, while a temperature of 120 °C was applied with inulin as wall material (Saéna et al. 2009). The wall material also affects the stability of polyphenols. Georgetti et al. (2008) reported that the degradation of polyphenol after spray drying was reduced in soybean extract when colloidal silicon dioxide (Tixosil 333) was added as a drying aid to the wall materials of maltodextrin and starch. Therefore, optimization of spray drying conditions, especially drying temperature and wall material, is critical to the success of polyphenol encapsulation.

10.3.3 Enzymes and Peptides

Enzymes and peptides are protein-based molecules that perform a variety of functions in organisms. Enzymes are capable of catalyzing one or more specific types of chemical or biochemical reactions, and have been used in a wide array of industrial applications, including cosmetics, textiles, feed, and food industries, and even for the production of fuel alcohol and organic synthesis (Kirk et al. 2002). Peptides consist of short amino acid sequences that have less intricate functionality than proteins, and are most often used in the pharmaceutical, biomedical (de la Rica and Matsui 2010), and food industries (Molina Ortiz et al. 2009; da Silva Malheiros et al. 2010). To maintain their viability during an extended shelf life and to protect their stability and bioactivity, enzymes are often encapsulated or immobilized for commercial applications (Gibbs et al. 1999). The encapsulation of peptides is performed mainly to attenuate their bitter taste (Molina Ortiz et al. 2009; Favaro-Trindade et al. 2010) and to protect their bioactivity (de la Rica and Matsui 2010). Spray dried enzymes include trypsin, amylases, proteases, glucose oxidase, pectinases, glucose isomerase, lactase, and pepsin (Bhandari et al. 2008), while spray dried peptides include casein hydrolysate (Molina Ortiz et al. 2009; Favaro-Trindade et al. 2010), nisin (de la Rica and Matsui 2010), capreomycin (Schoubben et al. 2010), and chicken meat protein hydrolysate (Kurozawa et al. 2011).

A key consideration in utilizing spray drying encapsulation of enzymes and peptides is how well these thermally labile materials resist heat denaturation by hot air. At the early stage of drying, when the droplet surface remains moisture-saturated (100 % relative humidity), its temperature is maintained at the wet-bulb temperature, which is significantly lower than the hot air temperature. As drying continues, the droplet temperature begins to rise as the diffusion of water to the droplet surface can no longer maintain 100 % moisture. At this stage, the protein is primarily in a solid state, and the surrounding air temperature decreases significantly due to moisture uptake (Ameri and Maa 2006). Thus, thermal denaturation is not typically observed in spray drying. In practice, the use of a lower inlet air temperature is advisable to reduce the potential thermal stress to the protein. However, protein denaturation typically occurs during dehydration, so the enzymes and peptides must be dried in the presence of wall materials and/or protective

materials such as lactose, sucrose, mannitol, gums, maltodextrins, or cyclodextrins (Daeman and van der Stage 1982). The types of protective agents added to wall materials can affect the activity of enzymes; for example, sucrose was reported to be more effective than trehalose in stabilizing lysozyme (Liao et al. 2002), but trehalose was found to be superior to mannitol, sucrose, and arginine hydrochloride as a stabilizer for spray drying of β-galactosidase (Broadhead et al. 1994). The authors suggest that sugars can protect lysozyme against dehydration stress by hydrogen bonding between the sugar and protein molecules.

Two other possible sources of stress during spray drying of enzymes and peptides are atomization and the air–water interface (Ameri and Maa 2006). Mathematical modeling estimated that the shear rate imparted on the protein from atomization is in the range of 10^4–10^5 s^{-1}, and proteins can sustain shear rates as high as 10^5 s^{-1} (Maa and Hsu 1996). Therefore, shear stress of this magnitude does not impart a significant stress to the enzymes and peptides. However, when shear stress is combined with air–water interface, it may cause significant aggregation for air–water interface-sensitive proteins such as recombinant human growth hormone (rhGH), bovine serum albumin (BSA), and lactate dehydrogenase (LDH) (Faldt and Berganstahl 1994; Maa and Hsu 1996; Adler and Lee 1999). Hence, it is suggested that comprehensive factors should be considered in spray drying of enzymes and peptides, such as relatively lower inlet and outlet air temperatures (typically 120–140 °C and 50–65 °C, respectively), suitable protective materials and total solids content (around 10–20 %), and low shear during atomization (Broadhead et al. 1992).

Most enzymes and peptides are amphiphilic compounds, and thus are susceptible to adsorption at the air–water droplet interface, where unusual surface energies may cause the protein to unfold, exposing hydrophobic regions to hot air (Ameri and Maa 2006). The unfolded protein may then undergo aggregation by the interaction of the exposed hydrophobic regions with other unfolded molecules until precipitation occurs. The enzymes and peptides on the powder particle surface may experience the most severe drying and shear-induced stress, and may degrade faster during long-term storage, primarily due to the partial loss of their secondary structure caused by temperature, moisture, and other environmental stresses related to drying. To mitigate surface denaturation, three options have been recommended for minimizing rhGH aggregation: the addition of a surfactant to prevent the formation of insoluble aggregates, the addition of divalent zinc ions to prevent the formation of soluble aggregates, and increasing the rhGH concentration in the liquid feed (Maa et al. 1998). The addition of a surfactant such as polysorbate 80 to the lactate dehydrogenase (Adler and Lee 1999) and Tween 80 to trypsin (Millqvist-Fureby et al. 1999) wall materials was reported to prevent enzyme inactivation during spray drying. The addition of surfactant is thought to be helpful for reducing the enzyme concentration on the particle surface, as the surfactant molecules are preferentially adsorbed at the air–liquid interface of the droplets, thus expelling proteins from the surface. The loss of enzyme and peptide activity can be estimated by incorporating the inactivation kinetics model with the drying kinetics model, because the inactivation rate is temperature/time- and moisture

content/time-dependent. Some existing inactivation kinetics models and relevant issues within the context of spray drying of food materials with bioactive compounds were recently reviewed by Chen and Patel (2007), and an appraisal showing the effect of surface and center water concentrations on the inactivation rate was proposed.

10.3.4 Vitamins

Vitamins are minor but essential constituents of foods. They are required for the normal growth, maintenance, and functioning of the human body, and insufficient supply can lead to many deficiency diseases, such as scurvy, pellagra, ariboflavinosis, dermatitis, and enteritis (Belitz et al. 2009). Hence, vitamin preservation during the processing and storage of foods is of critical importance. The use of multivitamin supplements has been reported to reduce the risk of certain diet-related disorders (Pocobelli et al. 2009).

Vitamins are usually divided into two general classes: fat-soluble vitamins, such as A (retinol and carotenoids), D (calciferol), E (α-tocopherol), and K (phytomenadione); and water-soluble vitamins, such as B1 (thiamine), B2 (riboflavin), B6 (pyridoxine), niacin, pantothenic acid, biotin, folic acid, B12 (cyanocobalamin), and C (L-ascorbic acid). However, vitamins are completely or partially deactivated or damaged during the cooking and processing of foods. The average loss of vitamins through processing/preservation of vegetables and fruits varies from 10 to 60 %, depending on the fruit and vegetable varieties and the techniques employed, with vitamin C, folic acid, and vitamin B6 generally less stable during high-temperature processing than vitamins A, B1, and B2, and niacin (Belitz et al. 2009). Micro- and nano-encapsulation, including spray drying, are good techniques for preserving vitamins (Murugesan and Orsat 2012). Due to their processing stability and commercial value, vitamins C and A (mainly carotenoids) are those most often used for spray drying encapsulation.

Microencapsulated ascorbic acid microparticles have been reported to prevent ascorbic acid color change, slow its core release rate, and generally mask its acidic taste (Uddin et al. 2001). Under spray drying inlet and outlet temperature parameters of 200–300 °C and 70–95 °C, respectively, the loss of ascorbic acid during encapsulation was found to be minimal (less than 2 %), and was much lower than with other encapsulation methods such as thermal phase separation, melt dispersion, and solvent evaporation (Uddin et al. 2001). The selection of a suitable encapsulation wall material is important for ensuring delivery of the ascorbic acid to the target organ for controlled release. For example, methacrylate copolymer of Eudragit® L is a pH-dependent enteric polymer composed of methacrylic acid–methacrylic acid methyl ester copolymers soluble from pH 6, which suggests that it is insoluble in the mouth and in the stomach, and soluble in the duodenum (pH around 6) (Weiss et al., 1993). Since the pH in the colon is around 7.5, researchers used Eudragit® L as a wall material in spray drying encapsulation for the delivery of

ascorbic acid to the lower part of the intestine and the jejunum and ileum (Esposito et al. 2002). The encapsulation of ascorbic acid with methacrylate showed very high efficiency, around 98–100 %, with good morphology and size distribution, although this was unable to slow the release of the drug with respect to the free form of ascorbic acid. In another study, in order to prepare drugs with sustained-release capability, vitamin C was encapsulated in tripolyphosphate (TPP)-cross-linked chitosan (TPP–chitosan) microspheres using spray drying (Desal and Park 2006). The authors found that an inlet temperature of 170 °C, liquid flow rate of 2 ml min^{-1}, and compressed air flow rate of 10 l min^{-1} provided optimal drying conditions, with encapsulation efficiency ranging from 45.7 to 68.7 %. The rate of vitamin C released from the TPP–chitosan microspheres decreased with increasing TPP solution volume and chitosan concentration (Desal and Park 2006). In these TPP–chitosan vitamin C microspheres, the molecular weight of chitosan also affected the encapsulation efficiency and controlled-release behavior, i.e. the encapsulation efficiency and release rate decreased with an increase in the chitosan molecular weight (Desal et al. 2006).

Spray drying is also a relatively effective method for encapsulation and preservation of carotenoids; one study reported that only 11 % degradation of carotene was observed in spray drying versus 14 % for drum drying (Desobry et al. 1997). Shu et al. (2006) also observed high encapsulation yield and efficiency, about 91 % and 82 %, respectively, in spray drying of lycopene. Because carotenoids are water insoluble, they were dissolved in oil phase to make an oil-in-water (O/W) and water-in-oil-in-water (W/O/W) multiple emulsions (Rodríguez-Huezo et al. 2004), or suspended in wall material solutions by homogenization (Desobry et al. 1997) before spray drying. Carotenoids of astaxanthin (Feldthusen et al. 2005), β-carotene (Loksuwan 2007), lutein (Reuscher et al. 2004), lycopene (Shu et al. 2006; Montenegro et al. 2007), and oleoresin (Rodríguez-Huezo et al. 2004; Santos et al. 2005), and samples containing carotenoids (Leuenberger et al. 2008) have been spray dried using encapsulating materials including gellan and mesquite gums, gum arabic, acacia gum, gelatin, skimmed milk, sodium caseinate, soy bean protein, trehalose, sucrose, lactose, maltodextrin, γ-cyclodextrin, pectin, cellulose, cellulose derivatives, modified starch, and modified polysaccharides, individually or in combination. Encapsulation yield and efficiency are dependent on the wall material formulation, core/wall material ratio, and homogenization pressure and temperature during the drying process (Shu et al. 2006).

10.3.5 Essential Fatty Acids

Essential fatty acids, such as ω-6 and ω-3 fatty acids, are those that humans and other animals require for good health but cannot synthesize in the body, and therefore must be ingested through the diet (Goodhart and Shils 1980). These fatty

acids, and especially the ω-3 fatty acids, are essential for normal growth and development, and may play an important role in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, other inflammatory and autoimmune disorders, and cancer (Simopoulos 1999). The ω-3 fatty acids in vegetable oils are primarily in the form of α-linolenic acid, with soybean oil and canola oil as important dietary sources (7–11 % of the total fat), whereas marine fish oils have a considerably higher content of ω-3 fatty acids (20–40 % of total fat), mostly in the form of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Alexander 1998). However, due to their highly unsaturated nature, the ω-3 fatty acids are very sensitive to lipid oxidation, which leads to a wide array of off-smells and off-tastes, varying from grass- and bean-like, to cardboard- and fish-like flavors (Beindorff and Zuidam 2010).

Encapsulation of essential fatty acids is a good method for slowing their oxidation, enhancing stability, controlling lipid-soluble flavor release, masking unpleasant taste and smell, and protecting dissolved substances against enzyme hydrolysis (Matsuno and Adachi 1993). However, the use of spray drying for encapsulation of fatty acids is a considerable challenge, as the spray drying process may induce oxidation due to the porous structure of the spray dried particles which allows high access of air to the oil, thus limiting the shelf life of the fatty acids (Hogan et al. 2003; Kolanowski et al. 2006, 2007). Therefore, the prevention of oxidation is of major concern for spray drying encapsulation of these highly unsaturated fatty acids. The extent of the protective effect is dependent on the nature of the wall materials, the fatty acids, and the encapsulation conditions (Matsuno and Adachi 1993). For example, the stability of fish oil was improved after spray drying in a polymer wall system of maltodextrin, modified starch, and whey protein concentrate (Jafari et al. 2008b), and good oxidation stability was reported for spray dried linoleic acid using whey protein concentrate as a wall material (Jimenez et al. 2004). In another study involving spray drying of linoleic acid, Minemoto et al. (2002) observed that wall material of gum arabic produced a better powder than maltodextrin in terms of encapsulation efficiency, stability, and oxidation resistance.

Lin et al. (1995) reported that the use of gelatin, sodium caseinate, and maltodextrin as wall materials for spray drying encapsulation was effective in improving the oxidative and thermal stability of crude squid oil, and that effectiveness was further enhanced by the addition of lecithin and carboxymethyl cellulose. Another study suggested that the stability of fish oil after spray drying was dependent on the interfacial composition and properties of both the oil–water interface in the parent emulsion and the surface composition of the drying droplets (Drusch et al. 2007). Emulsification of fish oil in a lecithin—chitosan multilayer system and the addition of EDTA were shown to have a positive effect on the stability of ω-3 fatty acids after spray drying (Shaw et al. 2007). The induction of simple coacervation by adding maltodextrin into the fish oil emulsion and hydroxypropyl methylcellulose (HPMC) solutions prior to spray drying was also found to increase oxidation stability (Wu et al. 2005). In addition, the esterification of polyunsaturated fatty acids with L-ascorbic acid and subsequent microencapsulation of the ester was demonstrated as a useful technology for slowing the

oxidation of linoleic acid (Jimenez et al. 2004). The particle size of the spray dried powder may also affect fatty acid stability; for example, it was noted that linoleic acid powder with larger particles was more stable during storage than that with smaller particles (Fang et al. 2005).

Other studies suggest that further improvement in the stability of encapsulated fatty acids may be achieved by conducting a Maillard reaction of wall materials of proteins (sodium caseinate, whey protein isolate, soy protein, or skim milk powder) with carbohydrates (glucose, dried glucose syrup, or oligosaccharides) at 60–100 °C for 30–60 min before spray drying, possibly because of changes in powder morphology and/or antioxidant effect of the Maillard products (Sanguansri and Augustin 2001; Augustin et al. 2006; Luff 2007). The addition of the antioxidants tocopherol or Trolox C to the oil prior to spray drying was also shown to improve the storage stability of spray dried fish oil (Hogan et al. 2003; Baik et al. 2004), although these compounds may act as pro-oxidants at high concentrations (Kolanowski et al. 2006). The use of trace metal chelation of citrem [citric acid esters of mono- and diglycerides of fatty acids] was found to improve the storage stability of the oil emulsion prior to spray drying but not after spray drying. The oil storage stability was significantly retarded by adding a combination of the antioxidants tocopherol, ascorbyl palmitate, and rosemary extract before the drying process (Serfert et al. 2009). In spray drying encapsulation of fatty acids, the fatty acid on the surface of particles may be released due to the rupture of the particle membranes that stabilizes them as an emulsion, which can result in high surface oil and free fat content in the dried powder. The released surface fat is prone to oxidation, as it is no longer encapsulated. Therefore, it is important to minimize the free fat content by selecting the correct drying conditions and formulation (Fang and Bhandari, 2012a).

10.4 Stability of Spray Dried Bioactives

Similar to other encapsulation processes, spray drying is not always efficient for the encapsulation of bioactives. Therefore, the un-encapsulated compounds, particularly those located on the powder particle surface, are prone to degradation during powder storage. Although spray drying conditions can be optimized to maximize encapsulation efficiency and retention of bioactive components upon drying, this may not necessarily result in good stability upon storage (Manojlović et al. 2010). Because of the small particle size and high surface area, spray dried carotenoid powder showed very fast degradation kinetics during storage (Desobry et al. 1997). Kolanowski et al. (2006, 2007) concluded that spray dried fish oil was even less stable against oxidation upon storage than fish oil, although this is not a common finding, and may be due to the drying conditions applied and/or materials used.

Generally, the storage stability of spray dried encapsulated bioactives is dependent on the formulation (e.g. wall and core materials, protective agents, emulsion type) before drying, operating conditions (e.g. inlet and outlet

temperature, nozzle type, air flow, feed speed) during drying, and packaging type and storage environment (light, humidity, and temperature) after the drying process. For example, the use of the relatively high-glass-transition-temperature (T_g) materials starch and β-cyclodextrin as encapsulating materials in spray drying delayed the degradation of ascorbic acid during storage and showed improved results over un-encapsulated ascorbic acid (Uddin et al. 2001). A formulation of lycopene-loaded O/W emulsion with gum arabic and sucrose mixture was demonstrated to slow the degradation of vitamins A and D3 during storage by 45 % compared with a formulation without lycopene (Montenegro et al. 2007). An air inlet temperature above 160 °C was observed to cause greater anthocyanin loss during spray drying and storage (Ersus and Yurdagel 2007). Interestingly, specific treatments of feed solutions before drying, such as the use of the Maillard reaction of protein–carbohydrate wall materials, has also been found to improve the storage stability of spray dried fish oil (Augustin et al. 2006) and ω-3 fatty acid (Luff 2007). The use of combined antioxidants (tocopherol, ascorbyl palmitate, and rosemary extract) before drying was reported to significantly improve the protective effects against autoxidation in spray dried fish oil during storage (Serfert et al. 2009). In addition, increasing the size of the emulsion and powder particles (e.g. 10–150 mm) may reduce the particle surface area and therefore improve storage stability (Soottitantawat et al. 2005).

Because most bioactive compounds are sensitive materials, the most common reactants must be avoided. These include adverse chemical, physical, and biological factors such as oxygen, moisture, light, elevated temperature, and microbial contamination (Morgan et al. 2006). The shelf life of spray dried fish oil (Kolanowski et al. 2007) and anaerobic probiotics of *Bifidobacterium* (Chávez and Ledeboer 2007) can be enhanced by packing them alone or in food powders under nitrogen or vacuum in metalized packaging material. The spray dried materials are believed to be more stable when stored at temperatures below their T_g, and lower storage temperature and water activity (a_w) generally lead to better stability, depending on the type of encapsulated core material. For example, the highest storage stability of spray dried bayberry polyphenols was found at 25 °C and with a_w of 0.33, and temperatures and a_w above this range caused faster polyphenol degradation and powder caking (Fig. 10.3) (Fang and Bhandari 2011). For spray dried enzymes and probiotics, one of the most important factors affecting their stability and activity during storage is residual moisture level, and the choice of optimal moisture content (on the order of 3–8 %) is a compromise between high survival rates immediately after drying (better survival with higher water content) and low rates of inactivation upon storage (better survival with lower water content, although not necessarily at 0 %) (Manojlović et al. 2010), such as moisture content of less than 5 % and a_w of less than 0.25 for storage of probiotics (Chávez and Ledeboer 2007). The addition of an appropriate amount of wall material is necessary to reduce the hygroscopicity and caking of encapsulated powders. After spray drying, further processing or coating (e.g. fluid bed coating and drying) may be necessary if a very high-quality

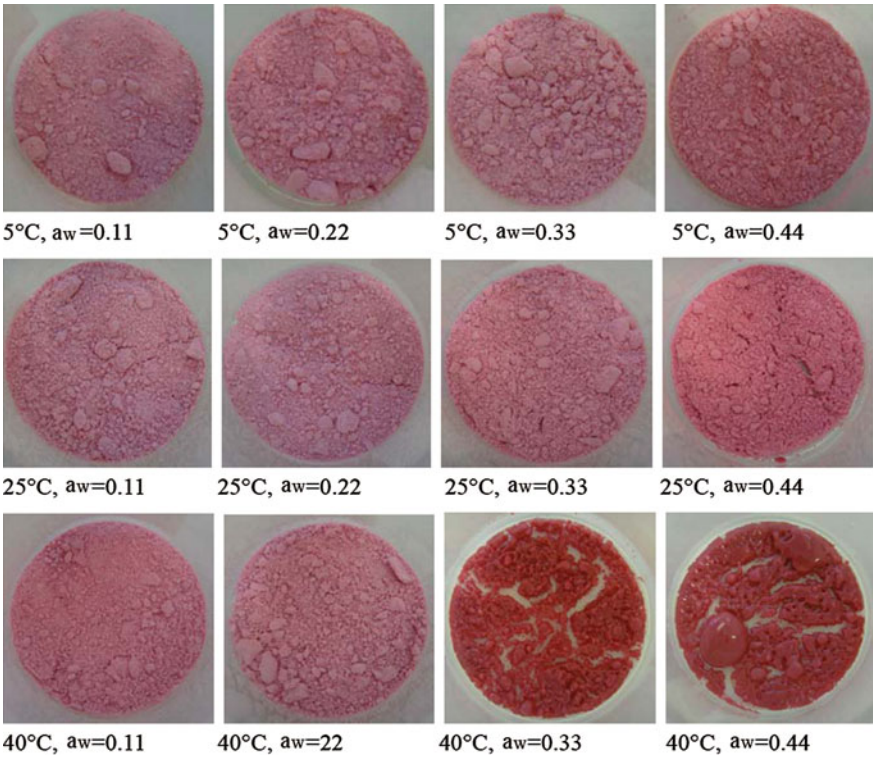

Fig. 10.3 Visual observations (digital camera photos) of spray dried bayberry polyphenol materials stored at different temperatures (5, 25, and 40 °C) and a_w (0.11–0.44) for 3 months (Fang and Bhandari 2011; with permission)

product is desired. Although it is possible to use very low temperatures for highly heat-sensitive bioactives, the process is not economically viable (Adhikari et al. 2009).

10.5 Conclusion and Future Research Opportunities

As discussed in other chapters of this book, a wide variety of techniques are available for the encapsulation and delivery of food bioactives, each with its own advantages and disadvantages. Spray drying represents a relatively simple continuous operation for producing food, nutraceutical, and pharmaceutical products with unique particle characteristics. Multiple factors must be optimized in spray drying encapsulation of bioactives, and this continues to be the subject of extensive research in order to manufacture high-value innovative products with extraordinary health benefits, in both the science and industrial sectors.

Research has shown that the spray drying step itself can be carried out successfully without difficulty, and can be optimized by trial-and-error procedures, but distinct improvements are needed in the choice of encapsulation materials to match the requirements of specific bioactive compounds (Gharsallaoui et al. 2007). The general requirements for wall materials are non-toxicity, low cost, bland taste, acceptable water solubility, good emulsion properties, and non-reactivity to core materials, as discussed in Sect. 2.1 and listed in Table 10.1. Because it is almost impossible for a single material to possess all of these properties, blends of multiple wall materials in one formulation are often applied. Therefore, numerous parameters within the formulation, including the type and concentration of wall polymer, core/wall ratio, type and concentration of heat/cold protectants, and type and concentration of emulsifier, may all contribute to the success of the encapsulation process (Fang and Bhandari 2012a). Each formulation is typically tailored to the specific type of core materials and specific spray drying conditions.

Innovation in spray drying technology is largely facilitated by combining existing novel technologies and the most recent knowledge of the processing and production sides (Bhandari et al. 2008). Several innovative designs and equipment modifications have been reported recently, including nano-spray drying (Lee et al. 2011), two-stage horizontal spray dryers (Huang and Mujumdar 2006), and spray-freeze drying (Maa et al. 1999). However, much work remains regarding product development, design quality, health and safety matters, environmental issues, and energy consumption. In addition, parameters and conditions in laboratory work should be fully tested and evaluated at pilot plants prior to large-scale production. Improvements in manufacturing technologies, new strategies for the stabilization of fragile bioactives, and the development of novel approaches will all contribute to the development of more efficient, economical, convenient, and simple spray drying techniques capable of satisfying demands across a wider range of industries for the manufacture of high-quality products.

References

Abee T, Wouters JA (1999) Microbial stress response in minimal processing. Int J Food Microbiol 50:65–91

Adhikari B, Howes T, Wood BJ, Bhandari BR (2009) The effect of low molecular weight surfactants and proteins on surface stickiness of sucrose during powder formation through spray drying. J Food Eng 94:135–143

Adler M, Lee G (1999) Stability and surface activity of lactate dehydrogenase in spray-dried trehalose. J Pharm Sci 88:99–208

Al C, Al T (2009) Ultrasonic vs. classical nozzles in probiotics encapsulation applications, XVIIth international conference on bioencapsulation. Groningen, The Netherlands; 24–26 Sept 2009. Poster P98, pp 1–4

Alexander JW (1998) Immunonutrition: the role of ω-3 fatty acids. Nutrition 14:627–633

Alvarez-Olmos MI, Oberhelman RA (2001) Probiotic agents and infectious diseases: a modern perspective on a traditional therapy. Clin Infect Dis 32:1567–1575

Ameri M, Maa YF (2006) Spray drying of biopharmaceuticals: stability and process considerations. Drying Technol 24:763–768
Anal AK, Singh H (2007) Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends Food Sci Technol 18:240–251
Ananta E, Volkert M, Knorr D (2005) Cellular injuries and storage stability of spray-dried Lactobacillus rhamnosus GG. Int Dairy J 15:399–409
Augustin MA, Sanguansri L, Bode O (2006) Maillard reaction products as encapsulants for fish oil powders. J Food Sci 71(2):E25–E32
Augustin MA, Hemar Y (2009) Nano- and micro-structured assemblies for encapsulation of food ingredients. Chem Soc Rev 38:902–912
Baik M-Y, Suhendro EL, Nawar WW, McClements DJ, Decker EA, Chinachoti P (2004) Effects of antioxidants and humidity on the oxidative stability of microencapsulated fish oil. J Am Oil Chem Soc 81:355–360
Beindorff CM, Zuidam NJ (2010) Microencapsulation of fish oil. In: Zuidam NJ, Shimoni E (eds) Encapsulation technologies for active food ingredients and food processing. Springer, New York, pp 161–185
Belitz HD, Grosch W, Schieberle P (2009) Food Chemistry. Springer, Berlin, pp 403–420
Bell LN (2001) Stability testing of nutraceuticals and functional foods. In: Wildman REC (ed) Handbook of nutraceuticals and functional foods. CRC Press, New York, pp 501–516
Bhandari B, Patel K, Chen XD (2008) Chapter 4. Spray drying of food materials: process and product characteristics. In: Chen XD, Mujumdar AS (eds) Drying technology in food processing. Blackwell, London, pp 113–159
Biesalski HK et al (2009) Bioactive compounds: definition and assessment of activity. Nutr 25:1202–1205
Bimbenet JJ, Bonazzi C, Dumoulin E (2002) Drying of foodstuffs. Drying. In: Proceeding of the 13th international drying symposium, pp 64–80
Broadhead J, Rouan SKE, Rhodes CT (1992) The spray drying of pharmaceuticals. Drug Dev Ind Pharm 18:1169–1206
Broadhead J, Rouan SKE, Hau I, Rhodes CT (1994) The effect of process and formulation variables on the properties of spray-dried β-galactosidase. J Pharm Pharmacol 46:458–467
Chávez BE, Ledeboer AM (2007) Drying of probiotics: optimization of formulation and process to enhance storage survival. Drying Technol 25:1193–1201
Chen XD, Patel KC (2007) Micro-organism inactivation during drying of small droplets or thin-layer slabs—a critical review of existing kinetics models and an appraisal of the drying rate dependent model. J Food Eng 82:1–10
Chiou D, Langrish TAG (2007) Development and characterisation of novel nutraceuticals with spray drying technology. J Food Eng 82:84–91
Corcoran BM, Ross RP, Fitzgerald GF, Stanton C (2004) Comparative survival of probiotic lactobacilli spray-dried in the presence of prebiotic substances. J Appl Microbiol 96:1024–1039
da Silva Malheiros P, Daroit DJ, Brandelli A (2010) Food applications of liposome-encapsulated antimicrobial peptides. Trends Food Sci Technol 6:284–292
Daeman ALH, van der Stege HJ (1982) The destruction of enzymes and bacteria during the spary drying of milk and whey. 2. The effect of the drying conditions. Neth Milk Dairy J 36:211–229
de la Rica R, Matsui H (2010) Applications of peptide and protein-based materials in bionanotechnology. Chem Soc Rev 39:3499–3509
de Vos P, Faas MM, Spasojevic M et al (2010) Encapsulation for preservation of functionality and targeted delivery of bioactive food components. Int Dairy J 20:292–302
Desai KGH, Park HJ (2005) Recent developments in microencapsulation of food ingredients. Drying Technol 23:1361–1394
Desal KG, Park HJ (2006) Effect of manufacturing parameters on the characteristics of vitamin C encapsulated tripolyphosphate-chitosan microspheres prepared by spray drying. J Microencapsulation 23:91–103
Desal KG, Liu C, Park HJ (2006) Characteristics of vitamin C encapsulated tripolyphosphate-chitosan microspheres as affected by chitosan molecular weight. J Microencapsulation 23:79–90

Desobry SA, Netto FM, Labuza TP (1997) Comparison of spray-drying, drum-drying and freeze-drying for β-carotene encapsulation and preservation. J Food Sci 62:1158–1162
Drusch S, Serfert Y, Scampicchio M, Schmidt-Hansberg B, Schwarz K (2007) Impact of physicochemical characteristics on the oxidative stability of fish oil microencapsulated by spray-drying. J Agric Food Chem 55:11044–11051
Dubernet C, Benoit JP (1986) La microencapsulation: Ses techniques et ses applications en biologie. L'actualité chimique, pp 19–28
Ersus S, Yurdagel U (2007) Microencapsulation of anthocyanin pigments of black carrot (*Daucus carota* L.) by spray drier. J Food Eng 80:805–812
Esposito E, Cervellati F, Menegatti E, Nastruzzi C, Cortesi R (2002) Spray dried Eudragit microparticles as encapsulation devices for vitamin C. Int J Pharm 242:329–334
Faldt P, Berganstahl B (1994) The surface composition of spray-dried protein-lactose powders. Colloids Surf 90:183–190
Fang X, Shima M, Adachi S (2005) Effects of drying conditions on the oxidation of lineoleic acid encapsulated with gum arabic by spray-drying. Food Sci Technol Res 11:380–384
Fang ZX, Bhandari B (2010) Encapsulation of polyphenols—a review. Trends Food Sci Technol 21:510–523
Fang ZX, Bhandari B (2011) Effect of spray drying and storage on the stability of bayberry polyphenols. Food Chem 129:1139–1147
Fang ZX, Bhandari B (2012a) Chapter 4, Spray drying, freeze drying and related processes for food ingredient and nutraceutical encapsulation. In: Garti N, McClements DJ (eds) Encapsulation technologies and delivery systems for food ingredients and nutraceuticals. Woodhead Publishing Ltd, Harvard, pp 73–109
Fang ZX, Bhandari B (2012b) Comparing the efficiency of protein and maltodextrin on spray drying of bayberry juice. Food Res Int 48:478–483
Fang ZX, Wang RB, Bhandari B (2013) Effects of type and concentration of proteins on the recovery of spray dried sucrose powder. Drying Technol 31:1643–1652
Favaro-Trindade CS, Santana AS, Monterrey-Quintero ES, Trindade, MA, Netto FM (2010) The use of spray drying technology to reduce bitter taste of casein hydrolysate. Food Hydrocolloid 24:336–340
Feldthusen JJ, Auweter H, Habich A, Lüddecke E, Pfeiffer A-M (2005) Method for producing dry powders of one or several carotenoids. WO 2005(075385):A2
Fleming RS (1921) The spray process of drying. J Ind Eng Chem 13:447–449
Fogler BB, Kleinschmidt RV (1938) Spray drying. Ind Eng Chem 30:1372–1384
Fritzen-Freire CB, Prudencio ES, Amboni RDMC, Pinto SS, Negrao-Murakami AN, Murakami FS (2011) Microencapsulation of *Bifidobacteria* by spray drying in the presence of prebiotics. Food Res Int 45:306–312
Fuller R (1992) History and development of probiotics. In: Fuller R (ed) probiotics, The scientific basis. Springer, Edinburgh, pp 1–8
Gardiner GE, O'Sullivan E, Kelly J, Auty MAE, Fitzgerald GF, Collins JK, Ross RP, Stanton C (2000) Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray drying. Appl Environ Microbiol 66:2605–2612
Georgetti SR, Casagrande R, Souza CRF, Oliveira WP, Fonseca MJV (2008) Spray drying of the soybean extract: effects on chemical properties and antioxidant activity. LWT—Food Sci Technol 41:1521–1527
Gharsallaoui A, Roudaut G, Chambin O et al (2007) Applications of spray-drying in microencapsulation of food ingredients: an overview. Food Res Int 40:1107–1121
Gibbs BF, Kermasha S, Alli I, Mulligan CN (1999) Encapsulation in the food industry: a review. Int J Food Sci Nutr 50:213–224
Goodhart RS, Shils ME (1980) Modern nutrition in health and disease (6th edn). Lea and Febinger, Philadelphia, pp 134–138
Haslam E, Lilley TH (1988) Natural astringency in foodstuffs: a molecular interpretation. Crit Rev Food Sci Nutr 27:1–40

Hogan SA, O'Riordan ED, O'Sullivan M (2003) Microencapsulation and oxidative stability of spray-dried fish oil emulsions. J Microencapsul 20:675–688
Huang L, Mujumdar AS (2006) Numerical study of two-stage horizontal spray dryers using computational fluid dynamics. Drying Technol 24:727–733
Jafari SM, Assadpoor E, He Y, Bhandari B (2008) Encapsulation efficiency of food flavours and oils during spray drying. Drying Technol 26:816–835
Jimenez M, García HS, Beristain CI (2004) Spray-drying microencapsulation and oxidative stability of conjugated linoleic acid. Eur Food Res Technol 219:588–592
Kailasapathy K, Chin JC (2000) Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. Immunol Cell Biol 78:80–88
Kirk O, Borchert TV, Fuglsang CC (2002) Industrial enzyme applications Curr Opin Biotechnol 13:345–351
Kitts DD, Weiler K (2003) Bioactive proteins and peptides from food sources. Applications of bioprocesses used in isolation and recovery. Curr Pharm Des 9:1309–1323
Kolanowski W, Jaworska D, Weissbrodt J, Kunz B (2007) Sensory assessment of microencapsulated fish oil powder. J Am Oil Chem Soc 84:37–45
Kolanowski W, Ziolkowski M, Weissbrodt J, Kunz B, Laufenberg G (2006) Micro encapsulation of fish oil by spray drying-impact on oxidative stability. Part I. Eur Food Res Technol 222:336–342
Kosaraju SL, D'ath L, Lawrence A (2006) Preparation and characterisation of chitosan microspheres for antioxidant delivery. Carbohydr Polym 64:163–167
Kosaraju SL, Labbett D, Emin M, Konczak I, Lundin L (2008) Delivering polyphenols for healthy ageing. Nutr Diet 65:S48–S52
Kris-Etherton PM et al (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 113:71–88
Kurozawa LE, Park KJ, Hubinger MD (2011) Spray drying of chicken meat protein hydrolysate: Influence of process conditions on powder property and dryer performance. Drying Technol 29:163–173
Lee SH, Heng D, Ng WK, Chan HK, Tan RBH (2011) Nano spray drying: a novel method for preparing protein nanoparticles for protein therapy. Int J Pharm 403:192–200
Leuenberger BH, Schlegel B, Voelker KM (2008) Process for the manufacture of a powder containing carotenoids. WO 2008(098694):A1
Lian WC, Hsiao HC, Chou CC (2002) Survival of bifidobacteria after spray-drying. Int J Food Microbiol 74:79–86
Liao Y-H, Brown MB, Nazir T, Quader JA, Marti GP (2002) Effects of sucrose and trehalose on the preservation of the native structure of spray-dried lysozyme. Pharm Res 19:1847–1853
Lievense LC, Van't Riet K (1994) Convective drying of bacteria. II. Factors influencing survival. Adv Biocheml Eng biotechnol 51:71–89
Lin CC, Lin SY, Hwang LS (1995) Microencapsulation of squid oil with hydrophilic macromolecules for oxidative and thermal stabilization. J Food Sci 60:36–39
Loksuwan J (2007) Characteristics of microencapsulated b-carotene formed by spray drying with modified tapioca starch, native tapioca starch and maltodextrin. Food Hydrocolloid 21:928–935
Ludescher RD, Shah NK, McCaul CP, Simon KV (2001) Beyond Tg: optical luminescence measurements of molecular mobility in amorphous solid foods. Food Hydrocolloid 15:331–339
Luff F (2007) Omega-3 and micro-encapsulation technology—making functional foods taste better for longer. Food Sci Technol 21:30–31
Maa YF, Hsu CC (1997) Protein denaturation by combined effect of shear and air-liquid interface. Biotechnol Bioeng 54:503–512
Maa YF, Hsu CC (1996) Effect of high shear on proteins. Biotechnol Bioeng 51:458–465
Maa Y-F, Nguyen P-A, Sweeney T, Shire SJ, Hsu CC (1999) Protein inhalation powders: spray drying vs spray freeze drying Pharma Res 16:249–254
Maa YF, Nguyen PA, Hsu CC (1998) Spray drying of air-sensitive recombinant human growth hormone. J Pharm Sci 87:152–159
Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L (2004) Polyphenols: food sources and bioavailability. Am J Clin Nutr 79:727–747

Manojlović V, Nedović VA, Kailasapathy K, Zuidam NJ (2010) Chapter 10 Encapsulation of probiotics for use in food products. In: Zuidam NJ, Shimoni E (eds) Encapsulation technologies for active food ingredients and food processing. Springer, New York, pp 269–302
Masters K (1991) The spray drying handbook. Longman Scientific and Technical, New York
Matsuno R, Adachi S (1993) Lipid encapsulation technology—techniques and applications to food. Trends Food Sci Technol 4:256–261
Mattila-Sandholm T, Myllarinen P, Crittenden R, Mogensen G, Fonden R, Saarela M (2002) Technological challenges for future probiotic foods. Int Dairy J 12:173–182
Mauriello G, Aponte M, Andolfi R, Moschetti G, Villani F (1999) Spray-drying of bacteriocin-producing lactic acid bacteria. J Food Prot 62:773–777
Millqvist-Fureby A, Malmsten M, Bergenstahl (1999) Spray-drying of trypsin-surface characterisation and activity preservation. Int J Pharm 188:243–253
Minemoto Y, Hakamata K, Adachi S, Matsuno R (2002) Oxidation of linoleic acid encapsulated with gum arabic or maltodextrin by spray-drying. J Microencapsulation 19:181–189
Molina Ortiz SE, Mauri A, Monterrey-Quintero ES, Trindade MA, Santana AS, Favaro-Trindade CS (2009) Production and properties of casein hydrolysate microencapsulated by spray drying with soybean protein isolate. LWT—Food Sci Technol 42:919–923
Montenegro MA, Nunes IL, Mercadante AZ, Borsarelli CD (2007) Photoprotection of vitamins in skimmed milk by an aqueous soluble lycopene-gum arabic microcapsule. J Agric Food Chem 55:323–329
Morgan C, Herman N, White P, Vesey G (2006) Preservation of microorganisms by drying: a review. J Microbiol Methods 66:183–193
Murugesan R, Orsat V (2012) Spray drying for the production of nutraceutical ingredients—a review. Food Bioprocess Technol 5:3–14
O'Riordan K, Andrews D, Buckle K, Conway P (2001) Evaluation of microencapsulation of a Bifidobacterium strain with starch as an approach to prolonging viability during storage. J Appl Microbiol 91:1059–1066
Peighambardoust SH, Tafti AG, Hesari J (2011) Application of spray drying for preservation of lactic acid starter cultures: a review. Trends Food Sci Technol 22:215–224
Percy S (1872) Improvement in drying and concentrating liquid substances by atomizing. US patent US125406 A
Pisecky J (1997) Handbook of milk powder manufacture. Niro A/S, Copenhagen
Pocobelli G, Peters U, Kristal AR, White E (2009) Use of supplements of multivitamins, vitamin C, and vitamin E in relation to mortality. Am J Epidemiol 170:472–483
Puri M, Sharma D, Barrow CJ (2012) Enzyme-assisted extraction of bioactives from plants. Trends Biotechnol 30:37–44
Ré MI (1998) Microencapsulation by spray drying. Drying Technol 16:1195–1236
Reineccius GA (2004) Spray-drying of food flavors. Drying Technol 22:1289–1324
Reuscher H, Kagan DI, Madhavi DL (2004) Coated carotenoid cyclodextrin complexes. US Patent 2004/0109920 A1
Riveros B, Ferrer J, Bórquez R (2009) Spray drying of a vaginal probiotic strain of Lactobacillus acidophilus. Drying Technol 27:123–132
Roberfroid MB (2000) Defining functional foods. In: Gibson GR, Williams CM (eds) Functional foods: concept to product. Woodhead Publishing Limited, Cambridge, pp 9–28
Robert P, Gorena T, Romero N, Sepulveda E, Chávez J, Saéna C (2010) Encapsulation of polyphenols and anthocyanins from pomegranate (*Punica granatum*) by spray drying. Int J Food Sci Technol 45:1386–1394
Rodríguez-Huezo ME, Pedroza-Islas R, Prado-Barragán LA, Beristain CI, Vernon-Carter EJ (2004) Microencapsulation by spray drying of multiple emulsions containing carotenoids. J Food Sci 69:E351–E359
Saéna C, Tapia S, Chávez J, Robert P (2009) Microencapsulation by spray drying of bioactive compounds from cactus pear (*Opuntia ficus-indica*). Food Chem 114:616–622
Sanguansri L, Augustin MA (2001) Encapsulation of food ingredients, WO2001074175

Santivarangkna C, Kulozik U, Foerst P (2007) Alternative drying processes for the industrial preservation of lactic acid starter cultures. Biotechnol Progr 23:302–315
Santos AB, Favaro-Trindade CS, Grosso CRF (2005) Preparation and characterization of paprika oleoresin microcapsules obtained by spray drying. Ciência e Tecnologia de Alimentos 25:322–326
Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L (2005) Dietary polyphenols and the prevention of diseases. Crit Rev Food Sci Nutr 45:287–306
Schmid K, Arpagaus C, Friess W (2011) Evaluation of the nano spray dryer B-90 for pharmaceutical applications. Pharm Dev Technol 16:287–294
Schoubben A, Blasi P, Giovagnoli S, Ricci M, Rossi C (2010) Simple and scalable method for peptide inhalable powder production. Eur J Pharm Sci 39:53–58
Serfert Y, Drusch S, Schwarz K (2009) Chemical stabilisation of oils rich in long-chain polyunsaturated fats during homogenisation, microencapsulation and storage. Food Chem 113:1106–1112
Shaw LA, McClements JD, Decker EA (2007) Spray-dried multilayered emulsions as a delivery method for ω-3 fatty acids into food systems. J Agric Food Chem 55:3112–3119
Shu B, Yu W, Zhao Y, Liu X (2006) Study on microencapsulation of lycopene by spray-drying. J Food Eng 76:664–669
Simopoulos AP (1999) Essential fatty acids in health and chronic disease. Am J Clin Nutr 70 (suppl):560S–5699S
Simpson PJ, Stanton C, Fitzgerald GF, Ross RP (2005) Intrinsic tolerance of bifidobacterium species to heat and oxygen and survival following spray drying and storage. J Appl Microbiol 99:493–501
Soottitantawat A, Bigeard F, Yoshii H, Furuta T, Ohkawara M, Linko P (2005) Influence of emulsion and powder size on the stability of encapsulated d-limonene by spray drying. Innovative Food Sci Emerg Technol 6:106–114
Surh YJ (2003) Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 3:768–780
To BCS, Etzel MR (1997) Spray drying, freeze drying, or freezing of three different lactic acid bacteria species. J Food Sci 62(576–578):585
Touhy KM, Probert HM, Smejkal CW, Gibson GR (2003) Using probiotics and prebiotics to improve gut health. Drug Discov Today 8:692–700
Uddin MS, Hawlader MNA, Zhu HJ (2001) Microencapsulation of ascorbic acid: effect of process variables on product characteristics. J Microencapsulation 18:199–209
Van de Guchte M, Serror P, Chervaux C, Smokvina T, Ehrlich SD, Maguin E (2002) Stress responses in lactic acid bacteria. Int J Gen Mol Microbiol 82:187–216
Villa-García M, Pedroza-Islas R, Moreno-Terrazas R, De La Rosa-Miranda M and Martínez-Ferez A (2010) Polidextrosa, inulina y aguamiel de maguey y su influencia en la sobrevivencia de Lactobacillus acidophilus microencapsulado en unamezcla de biopolimeros', Biop Mat-Biopolímeros: Fuentes, transformación, producción y plicaciones innovadoras. Fundación para la Education Superior Internacional, A. C. Electronic book access: www.fesi.org.mx
Vinatoru M (2001) An overview of the ultrasonically assisted extraction of bioactive principles from herbs. Ultrason Sonochem 8:303–313
Weiss G, Knoch A, Laicher A, Stanislaus F, Daniels R (1993) Microencapsulation of ibuprofen by a coacervation process using Eudragit L100-55 as an enteric polymer. Drug Dev Ind Pharm 19:2751–2764
Wu KJ, Chai XH, Chen Y (2005) Microencapsulation of fish oil by simple coacervation of hydroxypropyl methylcellulose. Chin J Chem 23:1569–1572
Zhang L, Mou D, Du Y (2007) Procyanidins: extraction and micro-encapsulation. J Sci Food Agric 87:2192–2197
Zuidam NJ, Shimoni E (2010) Overview of microencapsulates for use in food products or processes and methods to make them. In: Zuidam NJ, Shimoni E (eds) Encapsulation technologies for active food ingredients and food processing. Springer, New York, pp 3–29

Chapter 11
Protective Performance of Delivery Systems in Production, Shelf Life and Digestion

S. Drusch and R. Wilde

Abstract Protection is one of the major aims when designing delivery systems. Depending on the encapsulant, this may refer to minimisation of losses of volatile compounds, prevention of chemical deterioration or protection against an unintended release of the encapsulant. Specific examples are discussed in the present chapter. Important delivery systems are based on glassy carbohydrate-containing matrices prepared by spray drying, extrusion or spray granulation. These delivery systems provide excellent oxygen barrier properties. The impact of the molecular weight profile on molecular mobility and permeation of small molecules is discussed. The presence of low molecular weight carbohydrates generally improves the protective performance in the glassy state. A key characteristic in this context is the molecular free volume, which undergoes significant changes upon moisture sorption. Protective performance of hydrogel-based delivery systems, which are frequently used for immobilisation or in vivo delivery of an encapsulant, depends on diffusional phenomena and mechanical stability of the delivery systems. Since it becomes evident that protection is intrinsically tied to the structure of a delivery system, analytical methods to monitor the protective performance are reviewed.

Keywords Structure · Degradation · Release · Permeability · Alginate · Carbohydrate · Composition · Characterisation · Protective performance

11.1 Introduction

The development of delivery systems for bioactive ingredients in food science is closely linked to the state of the art in microencapsulation techniques. As outlined in several reviews during the last decades, the two major aims of microencapsulation are the protection of the encapsulated ingredient and/or its controlled release (Jackson and

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Lee 1991). In the very first pioneering years a wide range of substances were identified, for which microencapsulation may improve product performance. Among these substances were acids, bases, amino acids, colorants, enzymes, microorganisms, flavours, fats and oils, vitamins and minerals, salts, sweeteners and gases. The first applications were in the area of encapsulation of flavours and enzymes. In contrast, according to Jackson and Lee (1991) only a few examples and no industrial application of encapsulated microorganisms, minerals and vitamins were available, and thus the authors concluded that "the technology remains far from being fully exploited".

In the 1990s an increase in health consciousness of consumers became the driving force for the development of more healthy foods and functional foods. As reviewed by Schrooyen et al. (2001), development of these products presented a new challenge to food engineering. New ingredients had to be incorporated into foods, in which "they are slowly degraded, lose their activity or become hazardous by oxidation reactions. Ingredients can also react with components present in the food system, which may limit bioavailability, or change the colour or the taste of the product". The focus in the late 1990s was on new bioactive ingredients such as phytosterols, pro- and prebiotics, and new types of carotenoids and polyphenols, which would be available in purified form in the future and would thus require technological innovations to incorporate them into foods (Schrooyen et al. 2001). Also Gouin (2004) highlighted that microencapsulation was a niche market in the food industry. At the time, when Gouin (2004) was reviewing the available literature and the potential for encapsulation technologies in industrial applications in the food industry, apart from protective performance, the controlled release of the encapsulated ingredients came into focus and two years later Ubbink and Krüger (2006) stated that active ingredients of interest for the food industry "require innovative approaches because of the high sensitivity to a variety of physical and chemical factors". Controlled release includes issues related to protection, since the delivery system needs to protect the bioactive ingredient before it is released at the specific step in a process or a specific part of the gastrointestinal tract. Furthermore an uncontrolled release needs to be disclosed. Delivery systems are frequently based on pH- and time-dependent release principles. Due to unpredictable shifts in the pH and a wide variation in transit time, in vivo classical delivery systems frequently release their content in the upper part of the gastrointestinal tract with limited protection of the encapsulated bioactive ingredient (De Vos et al. 2010).

Microcapsules were initially classified into two different types: true microcapsules with a core-shell structure, and matrix capsules, in which the encapsulant is immobilised in a dispersed form. Nowadays, the structure of capsules is more complex, and a variety of systems have been developed for the incorporation of bioactive ingredients into liquid systems (Sagalowicz and Leser 2010). Keeping in mind the two main functions of protection and controlled release of the active ingredients, the term "delivery system" is considered to be more appropriate (Kosaraju 2005; Onwulata 2012).

Figure 11.1 summarises the main functions that delivery systems fulfil today. The most important aspects related to the stability of bioactive compounds/encapsulants are the physical loss, for example, of volatile aroma compounds, as well as chemical oxidation, enzymatic degradation and loss of viability in the case of microorganisms.

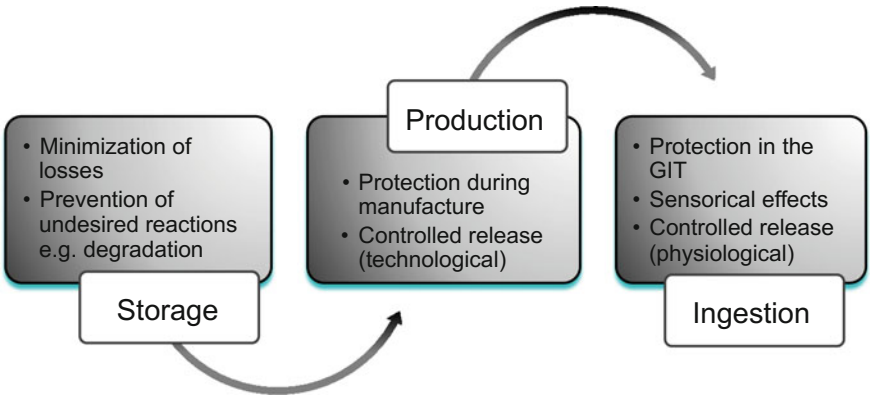

Fig. 11.1 Overview of the different functions of delivery systems in foods

Bioactive ingredients are frequently isolated compounds or mixtures of compounds that have been extracted from natural sources. In this purified form, they are particularly prone to adverse environmental conditions and thus need protection during storage. Food components may interfere with the activity of the bioactive ingredients, and undesired changes may occur during passage through the gastrointestinal tract. A controlled release may be desired during the process of food production, during ingestion to create sensorial effects or for physiological reasons in the gastrointestinal tract.

The aim of the present chapter is to give an overview on stability issues associated with active ingredients and to present current strategies for the design of delivery systems offering protection during storage, processing and digestion. In this context, materials science aspects and structure formation during production of the delivery system are of utmost importance, since they mainly determine the functionality.

11.2 Brief Overview of Stability Issues Related to Bioactive Components

Stability is one of the most critical issues when handling *flavouring agents*. Reineccius (2010) emphasised that different reasons for an undesirable change in flavour exist. Off-flavour may arise from chemical reactions including non-enzymatic browning, lipid oxidation, enzymatic reactions and/or light- or acid-catalysed reactions. Loss in flavour quality may also occur due to a loss of desirable flavour compounds that previously masked an off-flavour. One of the most frequently used groups of flavouring are citrus oils (Berger 2007). This group comprises oils produced from sweet orange (*Citrus sinensis*), bitter orange (*Citrus aurantium*), lemon (*Citrus lemon*), grapefruit (*Citrus paradise*), lime (*Citrus aurantifolia*) and mandarin (*Citrus reticulate*). Important chemical constituents with respect to flavour and off-flavour development in citrus oil are the monocyclic monoterpene limonene and the acyclic monoterpene-aldehyde citral. The latter comprises the two stereoisomers geranial and neral.

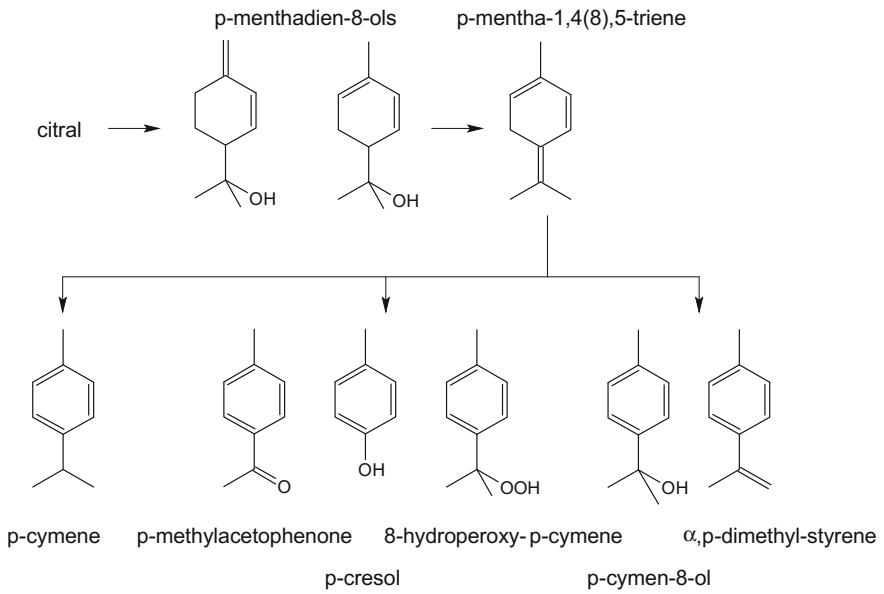

Fig. 11.2 Possible pathways of the degradation of citral in acidic environment

Fundamental work on the stability issues related to citral have been performed by Kimura et al. (1983) and Schieberle and Grosch (1988). Possible degradation pathways for citral in acidic environments are summarised in Fig. 11.2. These pathways include cyclisation with subsequent isomerisation or autoxidation, as well as dehydrogenation with subsequent hydration. Among others, the degradation products *p*-cresol and *p*-methylacetophenone are responsible for the development of phenolic and bitter almond-like odour (Ueno et al. 2006). Degradation of limonene, in contrast, is an autoxidation reaction with limonene oxide, carveol and carvacrol being important products of this process (Nguyen et al. 2009; Turek and Stintzing 2013). A physical loss is frequently caused by diffusion of the highly volatile flavour compounds from the food matrix or the delivery system. An excellent review on transport phenomena of volatile compounds was published by Cayot et al. (2008).

Unsaturated lipids undergo autoxidative deterioration during storage. This is a radical chain mechanism with three phases: initiation, propagation and termination. The autoxidation is initiated by free peroxy radicals formed in the food matrix, which cleave a hydrogen atom from the fatty acid molecule. Since the energy required for this reaction is lowest in a 1,4-pentadienic system as in polyunsaturated fatty acids, these fatty acids are particularly prone to oxidation. The reaction rate increases with the number of double bonds in the molecule. Taking linoleic acid as a reference, the relative rate of reaction for linolenic acid is 2.1, for arachidonic acid 2.9, and for docosahexaenoic acid 5.1 (Frankel 2005). The resulting fatty acid radicals are converted into monohydroperoxides. Through ß-elimination, carbonyl compounds like aldehydes, ketones, alcohols and low-molecular weight hydrocarbons are formed.

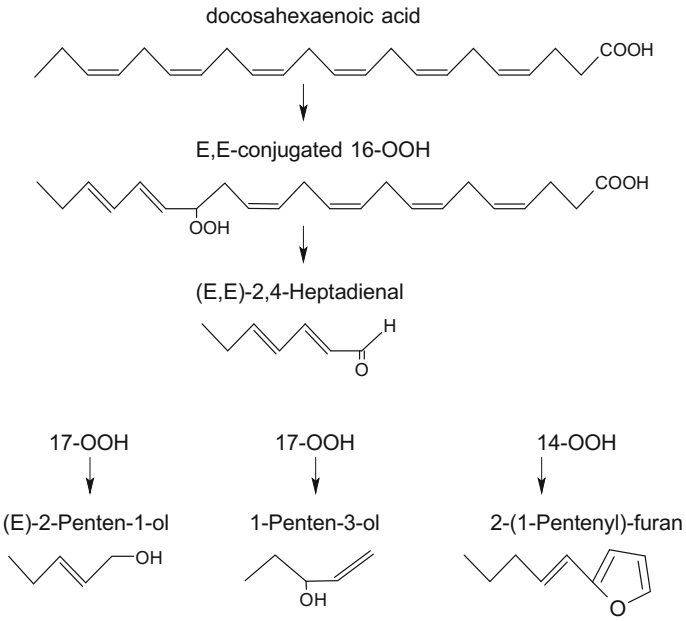

Fig. 11.3 Autoxidation of the polyunsaturated fatty acid docosahexaenoic acid

Figure 11.3 shows the most important steps in the autoxidation of docosahexaenoic acid. A wide range of substances resulting from the autoxidation of unsaturated fatty acids are aroma-active and contribute to off-flavour development in these products (Jacobsen 1999; Venkateshwarlu et al. 2004). Since the odour threshold is very low, protection against oxygen and a highly efficient encapsulation in the delivery system is a pre-requisite for stable products.

Within the very heterogeneous group of *vitamins*, the fat-soluble carotenoids, vitamins A, D and E, as well as the water-soluble vitamin B1, are prone to deterioration and may need to be protected during storage and processing of foods. Carotenoids may undergo isomerisation and fragmentation in absence of oxygen. In the presence of oxygen, oxidation occurs, which results in the formation of specific volatile aroma-active compounds. The impact of industrial processing on carotenoids has recently been summarised by Maiani et al. (2009). Tocopherols (vitamin E) are redox-active compounds and, thus, play a crucial role in stabilisation of lipid-containing foods. As a result of the reaction with a lipid peroxyl radical, a tocopheroxyl radical is formed. It is stabilised by delocalisation of the unpaired electron within the fully substituted chromanol ring system. The aim of encapsulation is to prevent oxidation of the tocopherols prior to their intended use as antioxidants. Within the group of water-soluble vitamins, vitamin B1 is rapidly degraded in the presence of nucleophilic substances and at elevated temperature. Protection against these factors is difficult to achieve and, as a consequence, there is hardly any literature available on encapsulation of vitamin B1.

Finally, there was a marked increase in encapsulation of microorganisms when nutritionists discovered the positive benefits of probiotic microorganisms. According to the World Health Organisation (WHO), probiotic microorganisms are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". In practice, probiotics commonly belong to the genera *Bifidobacterium* or *Lactobacillus*. As reviewed by Cook et al. (2012), after the administration of a probiotic, there is a considerable loss of viability as it passes through the low pH of the stomach and high bile salt conditions of the intestine. Such viability losses may be reduced by microencapsulating the bacteria in a polymer matrix. The latter must offer good protection against acidic pH and must be non-antimicrobial to ensure that the bacteria are not harmed by it.

11.3 Analytical Methods to Monitor Protective Performance

Analytical methods to evaluate the protective performance can be used at different stages in research and development or process control. At first, analysis of material properties and chemical structure of the constituents of the matrix of the formulation may provide data to estimate functionality. A qualitative description of the physical structure can be performed using different microscopic techniques. Scanning electron microscopy has become even more valuable with the development of environmental scanning electron microscopy, in which limitations of the high vacuum required for imaging have been overcome, and thus analysis of samples in their "wet" state is possible (James 2009). In addition, confocal laser scanning microscopy (CLSM) can be used to visualise the structure of the delivery system. In CLSM, the image is obtained from optical sections in the specific focal plane of the sample. The laser beam can penetrate the sample, and from multiple sections a three-dimensional image can be constructed (Lorén et al. 2007). The necessity of using a fluorescent dye may limit the application. Labelling of the matrix constituents may change the physical properties and thus affect the structure of the delivery system. Within the last decade, probe microscopy has become widely available for studying food structures down to the nanoscale. In atomic force microscopy (AFM), an image of the sample is produced by scanning the sample surface and texture with a sharp probe or a tip. The movement of the tip (fixed onto a cantilever) is monitored by a laser beam, and a change in its position tracks the motion of the cantilever (Morris 2007). AFM has been used to characterise inclusion complexes of functional ingredients with starch (Lesmes et al. 2009; Zabar et al. 2010) and structural aspects of emulsion-based delivery systems (Serfert et al. 2013).

Physical analysis of the structure includes determination of the density in the case of particle-based delivery systems, ranging from bulk density down to the true density as determined by gas displacement techniques. The surface area may be determined through nitrogen adsorption via the Brunauer–Emmett–Teller (BET) method, and

porosity can be analysed by mercury porosimetry or the BET method. In glassy polymer matrices, knowledge about free volume elements is of importance. These free volume elements are a conditional notion and are frequently described as cylindrical microcavities in the continuous phase of the amorphous matrix. Analysis of free volume elements may be performed using positron annihilation lifetime spectroscopy (PALS), inverse gas chromatography, ^{129}Xe nuclear magnetic resonance spectroscopy (NMR) or spin probe methods (Yampolskii and Shantarovich 2006). PALS is based on the measurement of the lifetime of positronium atoms, a bound state of positrons in a polymeric material. The positronium is trapped in the free volume elements and its lifetime depends on the size of these microcavities because of local differences in electron densities. Ubbink (2016) highlights that determination of the specific volume by gas displacement directly relates to the molecular hole volume.

Apart from the structure itself, it is worth investigating diffusion-related phenomena as a major cause of undesired reactions in delivery systems. These phenomena include the physical loss of volatile encapsulants as well as the diffusion of oxygen and other substances through the matrix of the delivery system, which result in undesirable changes in the composition of the encapsulant. Loss of volatiles is usually analysed by techniques that are generally used for the analysis of flavour in foods, i.e. gas chromatographic techniques. Depending on the type of analyte and its concentration of the volatiles, different trapping techniques and detection methods can be used (Ridgway et al. 2010; Balasubramanian and Panigrani 2011; Biniecka and Caroli 2011; Jeleń et al. 2012). In industrial quality control, these analyses might be supported by the use of sensors (Baldwin et al. 2011) or sensory evaluation by a sensory panel (Ross 2009; Tuorila and Monteleone 2009). Diffusion through the matrix of the delivery system may be analysed using spin probe techniques. One such method is electron spin resonance (ESR) oximetry. In general, ESR is based on the measurement of the absorption of energy from electromagnetic radiation by unpaired electrons according to their spin quantum number. For measurement of oxygen diffusion, a paramagnetic substance is incorporated in the matrix. Oxygen, as a paramagnetic substance, broadens the ESR signal of the probe through Heisenberg spin exchange (Andersen et al. 2000).

Routine techniques can be used to estimate the protective performance of the delivery system based on the reduction in encapsulant content over time in the delivery system or the supplemented food. Depending on the type of analyte, these techniques include colour measurement, photometric assays or chromatographic techniques such as high performance liquid chromatography, gas chromatography or headspace gas chromatography. Under some conditions it might be more appropriate to analyse the degradation products or the release of the encapsulant rather than encapsulant content in the delivery system. The reason is an increase in sensitivity. For example, off-flavour development resulting from oxidation of polyunsaturated fatty acids occurs far earlier than it will be detected by a decrease in the concentration of the fatty acids themselves. A major drawback is that these tests must be performed over a long period of time in order to evaluate the protective performance over the entire shelf life of a supplemented food. It is possible to run accelerated test protocols; however, the conditions have to be chosen very carefully

in order not to alter the physical structure or composition of the food, or to affect chemical reaction pathways and the order of the kinetics. For further reading on these topics, excellent publications are available (Kilcast and Subramaniam 2000; Steele 2000; van Boekel 2009).

Protective performance may also relate to controlling of the release of the encapsulant. The simplest approach for monitoring the performance of a delivery system in vivo is the use of in vitro test systems. These tests have been standardised for pharmaceuticals, which might be heavily affected by the composition of the body fluids in the gastrointestinal tract and the residence time, the appropriate device for facilitating agitation, and the duration of the sample in the test system. However, these methods need to be adapted prior to their use for encapsulated ingredients. It must also be taken into consideration that the encapsulant is ingested after it has been incorporated in a food matrix (Dressman et al. 1998; Galia et al. 1998). An overview of the composition of different media was recently provided by Marques et al. (2011). Since results from in vitro tests cannot precisely predict the protective effect of the delivery system in vivo or the release of the encapsulant, more sophisticated models have been developed to replace animal or human trials, which often face ethical and economic issues. As reviewed by Mackie (2012) "physically realistic" models have been developed in which mechanical forces and motility of the gastrointestinal tract are simulated in addition to variations in composition of the gastric and intestinal juice and the residence time.

11.4 Protective Performance of Glassy Carbohydrate-Based Matrices

It is general accepted and frequently mentioned in the literature that delivery systems based on inclusion of the bioactive ingredient in an amorphous carbohydrate-based matrix provides excellent protection against oxidative deterioration (Hogan et al. 2003; Gouin 2004; Reineccius 2010; Drusch et al. 2012). Possible techniques for preparation of these delivery systems include spray drying, spray granulation and extrusion, and a wide range of literature is available on the protective effect for flavouring agents and oils rich in polyunsaturated fatty acids. A review of this literature is beyond the scope of this chapter; for further reading some recent review articles are recommended (Madene et al. 2006; Vega 2006; Champagne and Fustier 2007; Drusch and Mannino 2009).

With respect to volatile bioactive ingredients like aroma-active compounds in flavouring agents, significant *losses* can occur *during production* of the delivery system. King (1995) highlighted that in spray-drying significant losses may already occur during atomisation. The reason is that there are substantial surface areas, turbulence and secondary flows within the sheets, ligaments and drops of liquids. Although most of the aroma-active ingredients are highly volatile and tend to be lost during evaporation of water from the drying droplet, the major proportion is retained

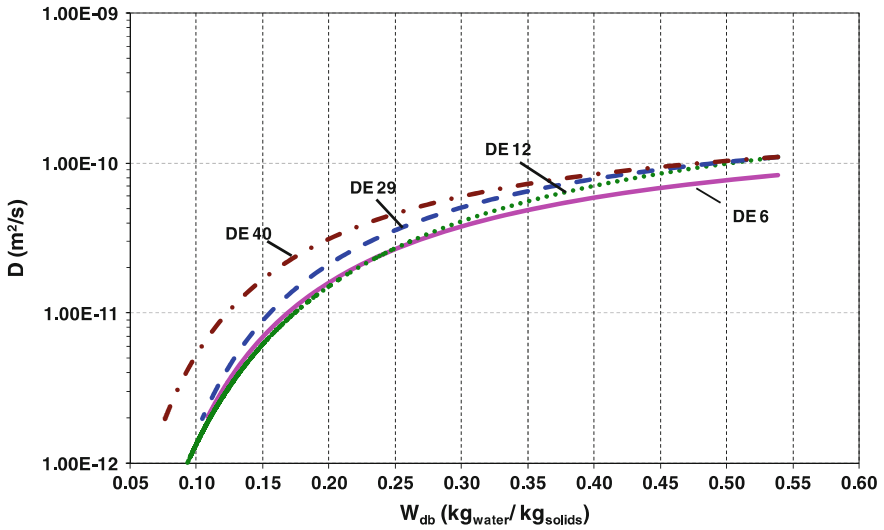

Fig. 11.4 Water diffusion coefficients at 70 °C of maltodextrin DE 6, 12, 29, and 40, calculated according to Yamamoto's model (Gianfrancesco et al. 2012)

inside the spray-dried particles. This can be explained by the *concept of selective diffusion*, which was developed by Rulkens and Thijssen (1972). The general background of this concept is the fact that the diffusion coefficient of water in the drying droplet decreases to a lower extent than the diffusion coefficient of volatile aroma compounds. Figure 11.4 shows the water diffusion coefficient of maltodextrin with different dextrose equivalents during drying at 70 °C (Gianfrancesco et al. 2012).

As a consequence, at high-solids content of the drying particle, the surface layer is still permeable to water, but not to the dissolved aroma-active compounds providing protection during the encapsulation process. Wang and Langrish (2009) reviewed the mechanisms leading to component segregation in spray-dried particles of multicomponent mixtures. According to their review, segregation is caused partly by differences in the diffusivity of the components. During drying, a crust is formed at the outer surface of the particle. Due to evaporation of water at the surface, local concentration of the individual compounds is increased, which causes diffusion of the components to the centre of the drying particle. This mechanism might also facilitate the encapsulation of bioactive ingredients.

During storage, tremendous differences can be observed in the *stability of the encapsulated bioactive ingredient,* depending on the matrix of the delivery system (Drusch et al. 2007). It is obvious that the protective effect of the delivery system is the result of a complex interplay governed by material science aspects of the matrix components and process conditions. In terms of both physical losses and oxidative deterioration, the micro- and submicron structure of the delivery system, responsible for the occurrence of diffusion phenomena, needs to be elucidated. Some examples to highlight this aspect are given below:

- Whorton and Reineccius (1995) described an increase in flavour retention with increased dextrose equivalent (DE) of the carrier matrix during spray drying and a superior oxidative stability at DE 36. As the authors state, at that time the reason for these observations was not well understood.
- Desobry et al. (1999) compared the stability of ß-carotene encapsulated in glucose syrup with a DE of 25 and with different molecular weight profile. Highest stability was achieved, when DE 25 was prepared from a blend of maltodextrin with DE 4 and glucose.
- Development of lipid hydroperoxide was retarded in spray-dried microcapsules when the DE was increased from 5.5 to 38 (Hogan et al. 2003).
- Drusch et al. (2009) described a significant difference in the development of lipid hydroperoxide content in microencapsulated fish oil during eight weeks of storage for a carbohydrate-based matrix with DE 50 compared with DE 38 and DE 18 (Fig. 11.5).

It is generally accepted that microstructural aspects as determined by particle size, surface area, microporosity and density have a significant effect on the functionality of delivery systems. However, in the study by Drusch et al. (2009), for the first time the protective effect of the capsule matrix was underlined by analysis of structural characteristics in the submicron range. Based on microstructural parameters, all carbohydrate-based delivery systems showed a similar structure, but PALS revealed that differences in the size of free volume elements in the amorphous carbohydrate-based matrices existed, depending on the DE, and thus the molecular weight profile (Drusch et al. 2009).

A low free volume in the matrix of the delivery system is related to a low oxygen permeability, since it suppresses the diffusion of small permeants through the matrix

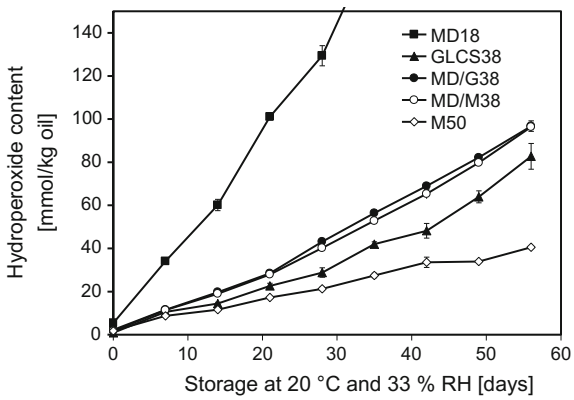

Fig. 11.5 Development of the hydroperoxide content of fish oil microencapsulated into matrices with different molecular weight profiles upon storage at 20 °C and 33 % relative humidity; M50: maltose, GLCS38: glucose syrup with DE 38, MD/G38: blend of maltodextrin with DE 18 and glucose with a final DE of 38, MD/M38: blend of maltodextrin with DE 18 and maltose with a final DE of 38, MD18: maltodextrin with DE 18 (Stephan Drusch et al. 2009)

(Edlund et al. 2012). Permeation of gases like oxygen or nitrogen is governed by a coupled mechanism of dissolution and diffusion as described in the *solution-diffusion model*. It can be characterised by the permeability coefficient P [kg m^{-1} s^{-1} Pa^{-1}]:

$$P = D * S \tag{11.1}$$

where D is the diffusion constant and S is the solubility. The penetrating molecules dissolve in the matrix and subsequently undergo molecular diffusion, driven for example by a concentration or pressure gradient. The solubility itself includes two phenomena, the dissolution of the gas in the matrix and the adsorption in pre-existing voids.

In the glassy state, the variation in solubility turns out to be much weaker than the variations in the diffusion coefficient. As a consequence, parameters affecting the rate of diffusion govern the rate of permeation. The concentration of the penetrant itself is negligible, since for most gases only weak interactions with the matrix occur, and diffusion can be described by Fick's laws:

$$J_x = -D * \frac{\partial C}{\partial x} \quad \text{(First law of Fick)} \tag{11.2}$$

where J_x is the diffusion flux [mol m^{-2} s^{-1}], D is the diffusion coefficient [m^2 s^{-1}], C is the concentration [mol m^{-3}] and x is the position/length [m].

$$\frac{\partial C}{\partial t} = -D * \frac{\partial^2 C}{\partial x^2} \tag{11.3}$$

where t is the time [s]. The diffusion coefficient depends on temperature, and its development follows the Arrhenius law:

$$D = D_0 * e^{\frac{-E_D}{RT}} \tag{11.4}$$

where D_0 is a constant, E_D is the activation energy for diffusion, R is the universal gas constant and T is the temperature.

The process of diffusion is considered to be a jump-process in which the penetrant must jump from one region of free volume to the other. Schoonman et al. (2002) postulated that a direct relation between porosity and gas entrapment capacity existed, as well as an effect of porosity on the diffusion of low molecular weight penetrates. Porosity comprises open pores as well as voids and free volume elements, thus covering a wide size range from micropores to sub-micron pores (Falco et al. 2013). Glassy carbohydrates have a low fraction of free volume and show very restricted chain motion. Thus, the parameter of interest is the energy is required to separate molecule chains so that a void is formed, which allows the penetrant to jump from one equilibrium position to another. Meares (1954) proposed a molecular model for the diffusion of gas molecules in which this energy was calculated from the work against the internal pressure:

$$E_D = \int^{V} p * \partial V = (\pi/4) d^2 N_A \lambda \varepsilon \tag{11.5}$$

where E_D is the activation energy for diffusion, p is the pressure, V is the volume, d is the penetrant diameter, N_A is the Avogadro number, λ is the cylindrical void jump length and ε is the cohesive energy density. Though this was one of the first molecular models to describe diffusion in polymers, and more sophisticated models have been developed, the model is still used to provide a general understanding. With respect to the molecular network in the glassy matrix, internal pressure is related to the cohesive energy density, which is relatively large for maltodextrins. According to Falco et al. (2013), these jumps are rare in glassy systems and correlate with the size of the penetrant and the physical state of the matrix.

Data from the literature also support that the molecular model can describe the diffusion of oxygen through glassy matrices as determined by ESR, since it was observed that this is an activated process (Andersen et al. 2000). The energy barrier, which needs to be overcome is due to straining forces induced by the molecular potential. For theoretical calculation, the transition state theory applies, but its detailed description goes beyond the scope of this article. An excellent overview of molecular models of diffusion, free volume models and their combination has been provided by Yampolskii et al. (2006).

Ubbink and co-workers performed several studies on the impact of the molecular weight profile of carbohydrates, temperature and water on structural and thermodynamic aspects of glassy carbohydrate-based matrices. These studies represent the current state of knowledge, explaining in an interdisciplinary approach the results of the examples given at the beginning of this subchapter, and have recently been summarised by Ubbink (2016). In the glassy state at low water content, the hole volume of the carrier matrix decreases with increasing fraction of low molecular weight carbohydrate content, namely maltose. Low molecular weight carbohydrates act as a plasticiser by reducing the molecular entanglement of polymer chains, and thus enable molecular reorganization under conditions where the polymer chains would be in the glassy state. An improved molecular packaging leads to higher matrix density and reduced mobility of small permeants in matrices containing low molecular weight carbohydrates (Kilburn et al. 2005). Concerning the effect of moisture sorption and moisture content on structural changes in the carrier matrix, Townrow et al. (2010) define different phases. At very low water content, water molecules preferentially bind to the carbohydrate chains. Initially it was hypothesised that water is preferentially located in free volume holes between the carbohydrate molecules (Townrow et al. 2007), and thus its role was described as a "hole-filler" and an "anti-plasticiser". Some years later, Townrow et al. (2010) showed that most of the water molecules were not located in the holes, but were molecularly dispersed and bound to carbohydrates, most likely occupying positions on the edges of holes. Minimum hole volume occurred at water content between 4 and 7 % (Ubbink 2016). Above this level, the well-known plasticizing effect of water occurs. It is worth mentioning that hole size expansion does occur already in

the glassy state. Therefore, determination of the glass transition temperature alone may not be sufficient to draw conclusions on matrix permeability and matrix mobility. Local properties at the molecular and submolecular levels must also be considered. Finally, in the rubbery state, molecular weight has no effect on hole size (Townrow et al. 2010).

Concerning **physical losses of flavour compounds**, diffusion in motionless systems like encapsulated ingredients can also be described by Fick's laws. As reviewed by Cayot et al. (2008), diffusion coefficients in porous solids are significantly lower than in aqueous systems or water (Fig. 11.6). Interactions of the flavour compounds with matrix constituents, particularly during preparation of the delivery systems, must be considered.

The effect of the preparation process of a carbohydrate-based delivery system on the resulting structure is not well investigated. As shown in Fig. 11.7, flavour loss is significantly lower in a delivery systems prepared via extrusion than in systems prepared by spray-drying or spray granulation (Uhlemann and Reiß 2010). In comparing the oxidative stability of encapsulated polyunsaturated fatty acids, delivery systems prepared by spray granulation provided significantly higher protection than those prepared by spray drying (Anwar and Kunz 2011). The latter can be attributed to differences in the surface oil content and possibly to differences in air inclusion within the system. However, structural differences between extruded and spray-granulated delivery systems need to be investigated.

Finally, phase transition strongly affects the protective performance of an amorphous carbohydrate-based delivery system. Changes in the structure of the delivery system such as recrystallisation result in a release of the entrapped encapsulant or accelerated oxidation due to a higher rate of oxygen diffusion (Drusch et al. 2006). The impact of glass transition on food processing was recently reviewed by Roos (2010).

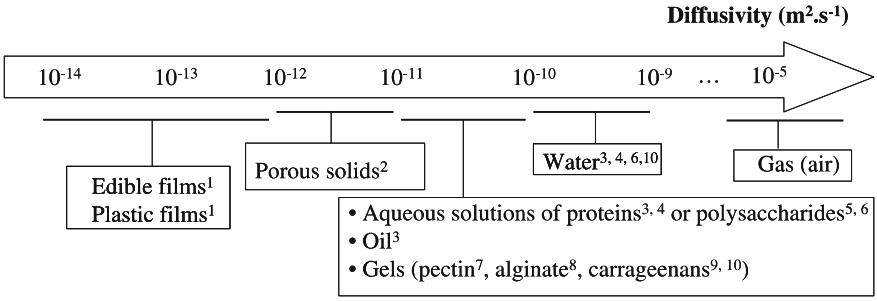

Fig. 11.6 Diffusion coefficient of flavour compounds in different matrices (reprinted with permission from Cayot et al. 2008; refer to this manuscript to see all references for the figure)

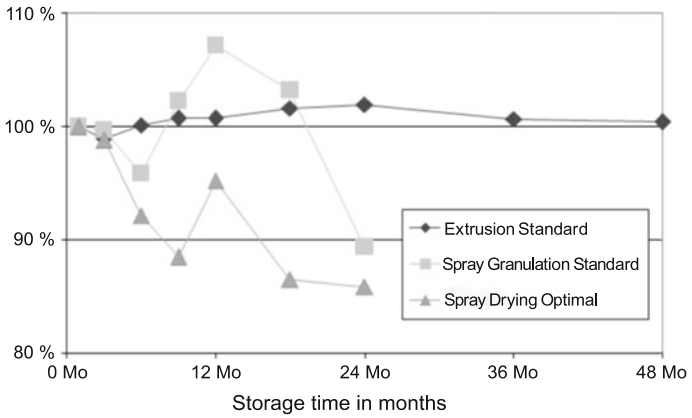

Fig. 11.7 Flavour retention in sugar glass measured as the residual percent of limonene at 40 °C (reprinted with permission from Uhlemann and Reiss 2010)

11.5 Protective Performance of Hydrogel-Based Capsules

Capsules consisting of gelled hydrocolloids have already been examined with respect to their protective performance, shelf life and digestion and are frequently used in the pharmaceutical or food industry. Generally, polysaccharide and protein hydrogels are used as immobilization matrices and protective structures for sensitive materials such as pharmaceuticals, foods and agricultural products (Kuang et al. 2010; Borgogna et al. 2011). The most common encapsulants are living cells and microorganisms, which include probiotics, drugs and pharmaceuticals, enzymes, oil-soluble flavours and vitamins, nutritional oils and fatty acids. A general overview of encapsulation of probiotic bacteria was provided by Kailasapathy (2002) and Anal and Singh (2007). Commonly used polysaccharides or proteins are derived from plants, bacteria, fungi, algae or animals. The number of polysaccharides investigated for technological applications of hydrogels is extensive (Coviello et al. 2007). For encapsulation purposes, cellulose, starch and pectin are the most commonly used polysaccharides obtained from higher plants, whereas alginate, carrageenan and agar from seaweed are well-known gelling polymers for hydrogel-based capsules (Blandino et al. 1999; Coviello et al. 2007; Faroongsarng and Sukonrat 2008; Rinaudo 2008; Cook et al. 2012; Gombotz and Wee 2012). In addition, chitosan from fungi or animal sources (crustaceans), gelatine, proteins obtained from various animal by-products, and gellan gum from microorganisms have been proposed for various applications (Murano 1998; Prüße et al. 2000; Coviello et al. 2007). Gelatine was also found to be suitable as encapsulation material for low-moisture or oil-phase food ingredients and pharmaceuticals (Bourtoom 2008).

With the use of hydrogel-based capsules, a controlled, sustained or timed release is possible (Kuang et al. 2010). Therefore, protective performance can be understood as the capability to prevent unintended leakage of the encapsulant into the

surrounding environment. As an example, probiotic bacteria or other microorganisms should preferably be released in the colon after ingestion of a food item. Therefore, the capsule needs to be acid-stable, and its integrity must not be affected by certain enzymes in the upper gastrointestinal tract, but should be dissolved in the presence of enzymes of the colon. Anal and Singh (2007) and Gombotz and Wee (2012) described three mechanisms of release for this type of capsule: (1) diffusion through the pores of the polymer network, (2) breakage of the capsule shell by mechanical stress or thermal treatment and (3) dissolution of the capsule shell by enzymatic activity or pH changes. In a hydrophilic matrix, the release of the encapsulated molecules occurs mainly by diffusion across the hydrated gel layer of swelling polymers. The release by diffusion and surface erosion has been found to occur frequently in hydrophobic matrix systems (Kuang et al. 2010).

Hydrogel-based capsules, consisting of polysaccharides and proteins, are generally very hydrophilic and have *poor water and gas barrier properties* (Kosaraju 2005; Rhim and Ng 2007; Bourtoom 2008; Borgogna et al. 2011). For example, the pore size of alginate beads ranges from 5 to 200 nm (Martinsen et al. 1991). Due to the pore size, the above mentioned diffusion rates are very high (Gombotz and Wee 2012). The diffusion of small molecules, such as oligosaccharides, amino acids, water and ethanol, is not affected by the alginate gel matrix (Tanaka et al. 1984; Chai et al. 2004). These molecules can freely diffuse through the pores of the alginate gel matrix. The diffusion of larger molecules, such as proteins, enzymes or fats, depends on their molecular weight and charge, type and concentration of alginate, and the resulting gel composition as well as the gelling process (Martinsen et al. 1991). A net positive charge inhibits the diffusion from the gelled matrix because of electrostatic interactions with the negatively charged alginate polymer (Smidsrød and Skjåk-Braek 1990).

Hydrogel-based capsules are highly suitable for applications in which immobilisation of a techno-functional catalytic additive such as an enzyme or a microorganism is desired. The porosity permits high diffusion rates for substrates and products. Nevertheless, the controlled release (and thus protection against unintended release) of molecules such as proteins or enzymes from hydrogel beads is difficult because of the wide range of pore size distribution (Smidsrød and Skjåk-Braek 1990). The pore structures of alginate gel matrices differ based on the source of raw materials and the chemical composition of the alginate (Gombotz and Wee 2012). Gels of alginates with high α-L-guluronic acid content have an open pore structure, whereas gels of alginates with higher β-D-mannuronic acid content are less porous. In order to control the release through the encapsulating material, the alginate gel matrices should have a defined pore size and a narrow pore size distribution. The pore volume fraction and inter-pore connections are also important factors influencing the release of molecules, and depend on the composition and crosslink density of the hydrogel polymer network (Hoffman 2012). In addition, the molecular weight determines the physical properties of the alginate: the higher the molecular weight, the lower the diffusion rate (Murata et al. 1993).

In order to modify the barrier properties of the alginate matrix, various additives have been included. In particular, other polysaccharides (e.g. chitosan,

tripolyphosphate [TTP]-chitosan, cellulose derivatives, pectin, starch, polyethylene glycol [PEG]), proteins (e.g. gelatine, poly-L-lysin) or lipids (e.g. beeswax, carnauba wax, shellac, vegetable oil, palm oil) have been used (Table 11.1). If positively charged additives (e.g. poly-L-lysin, gelatine, chitosan) are used in combination with negatively charged alginate molecules, the diffusion of larger molecules can be prevented (George and Abraham 2006) due to polyionic complexation by electrostatic interactions, resulting in the formation of insoluble capsules with smaller average pore sizes (Coviello et al. 2007).

Due to their hydrophobic properties, lipids as well as resins and waxes show good water vapour barrier characteristics and may be used in combination with proteins or polysaccharides. However, sensory properties such as greasy surfaces, waxy taste or rancidity in the fats restrict their use in the food industry (Rhim and Ng 2007). Moreover, capsules containing excessive amounts of lipids show low mechanical and limited oxygen barrier properties, inferior to those consisting of

Table 11.1 Overview of commonly used additives for preparation of alginate-based hydrogel capsules

Pectin	Liu and Krishnan (1999), Silva et al. (2009), Sandoval-Castilla et al. (2010), Wang et al. (2013)
Carboxymethyl cellulose	Blandino et al. (1999)
Hydroxypropyl cellulose	Karewicz et al. (2010)
Hydroxypropyl methylcellulose	Lee et al. (2003), Borgogna et al. (2010), Bellich et al. (2011)
Ethyl cellulose	Bodmeier and Wang (1993)
Microcrystalline cellulose	Dogan and McHugh (2007), Bilbao-Sáinz et al. (2010)
Chitosan	Gåserød et al. (1998, 1999), Sezer and Akbuga (1999), Lee et al. (2003), Chen et al. (2006), Kim et al. (2008), Bellich et al. (2011)
TTP chitosan	De Moura et al. (2009)
Starch	Singh et al. (2009), Tan et al. (2009), López-Córdoba et al. (2014)
Polyethylene glycol	Koyama and Seki (2004), Mahou and Wandrey (2010), Esser and Tessmar (2013)
Gelatine	Almeida and Almeida (2004), Dong et al. (2006), Li et al. (2009), Saravanan and Rao (2010)
Poly-L-lysin	Liu and Krishnan (1999), Ferreiro and Tillman (2002), Leick et al. (2011)
Wax (bee)	Habig-McHughet al. (1993), Hambleton et al. (2012)
Palm oil	Hansen et al. (2002)
Shellac	Qussi and Suess (2005), Henning et al. (2012), Leick et al. (2011)
Whey proteins	Chen and Subirade (2006), Wichchukit et al. (2013)

CMC carboxymethyl cellulose, *HPC* hydroxypropyl cellulose, *HPMC* hydroxypropyl methyl cellulose, *EC* ethyl cellulose, *MCC* microcrystalline cellulose, *TTP* tripolyphosphate, *PEG* polyethylene glycol

proteins and polysaccharides. By combining various polysaccharides, proteins and lipids, the protective performance, e.g. mechanical and barrier properties, can be improved (Falguera et al. 2011). Finally, the diffusion of large molecules can also be minimised by partial drying or by pH reduction (Smidsrød et al. 1973). By drying alginate capsules, the alginate concentration inside the capsule rises, which leads to a reduction of the average pore size (Smidsrød and Skjåk-Braek 1990).

In contrast to the limitations in protective performance against diffusion-related phenomena, hydrogel capsules show excellent mechanical and structural properties, which gains importance in *protection against unintended release* via *erosion* of the capsule (Murano 1998; Falguera et al. 2011). The capsule shell must be thermally and mechanically stable and display a certain degree of elasticity and plasticity (Borgogna et al. 2011). The physical properties depend on the composition, sequential structure and molecular size of the polymer (Smidsrød et al. 1973) as well as on the gelling process and the application of additives. Alginate-based capsules with the highest mechanical strength were made from alginates with an α-L-guluronic acid content greater than 70 % and an average length of the α-L-guluronic acid blocks of more than 15 units (Martinsen et al. 1989; Gombotz and Wee 2012). With increasing molecular weight, the gel strength increases (Kuo and Ma 2001). For a molecular weight higher than 2.4×10^5, the gel strength is independent from the molecular weight (Martinsen et al. 1991). In addition to the raw material, the gelling process is crucial for the mechanical strength of the hydrogel-based capsules. It is important to control it by specifying the alginate and calcium concentrations, the temperature and the pH-value of the system (Martinsen et al. 1989; Roopa and Bhattacharya 2008). Over a temperature range from 0 to 100 °C, alginate forms thermostable gels with properties largely dependent on the characteristics of the polymer and the gelling process (Gombotz and Wee 2012). With alginate concentrations below 0.5 %, the gel exhibits properties of both solids and liquids, and low gel strength (Roopa and Bhattacharya 2008). Again, this concentration is highly dependent on the structure of the alginate. The authors also observed that the mechanical stability of the polymer gel network decreased with increasing pH (Roopa and Bhattacharya 2008). Others have shown that the use of different additives such as proteins or polysaccharides, especially nanocomposites (e.g. TTP-chitosan, microcrystalline cellulose [MCC]) or positively charged molecules (e.g. poly-L-lysin, gelatine, chitosan), can increase the physical stability (Falguera et al. 2011).

In summary, there is a wide range of factors affecting the protective performance of hydrogel capsules, thus providing opportunities for modifying the controlled release of large molecules. Additionally, mechanical and chemical stability can be modified by means of suitable additives and by controlling the process of capsule preparation. With respect to preventing diffusion of small molecules through the capsule membrane pores, no promising additives have been identified to date, and the possibilities for modification are limited (Chan and Neufeld 2010). The pore size and the resulting diffusion process are only partially reduced. A future

challenge will be to combine formulation and process technology in order to prepare hydrogel-based capsules with a controlled protective performance against diffusion of low molecular weight constituents.

References

Almeida PF, Almeida AJ (2004) Cross-linked alginate-gelatine beads: a new matrix for controlled release of pindolol. J Controlled Release 97:431–439

Anal AK, Singh H (2007) Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends Food Sci Technol 18:240–251

Andersen AB, Risbo J, Andersen ML, Skibsted LH (2000) Oxygen permeation through oil-encapsulating glassy food matrix studied by ESR line broadening using a nitroxyl spin probe. Food Chem 70(4):499–508

Anwar SH, Kunz B (2011) The influence of drying methods on the stabilization of fish oil microcapsules: comparison of spray granulation, spray drying, and freeze drying. J Food Eng 105(2):367–378

Balasubramanian S, Panigrani S (2011) Solid phase microextraction (SPME) techniques for quality characterization of food products: a review. Food Bioprocess Technol 4:1–26

Baldwin EA, Bai J, Plotto A, Dea S (2011) Electronic noses and tongues: applications for the food and pharmaceutical industries. Sensors (Basel, Switzerland) 11(5):4744–4766

Bellich B, Borgogna M, Cok M, Cesàro A (2011) Release properties of hydrogels: water evaporation from alginate gel beads. Food Biophys 6(2):259–266

Berger RG (2007) Flavours and fragrances. Springer, New York

Bilbao-Sáinz C, Avena-Bustillos RJ, Wood DF, Williams TG, McHugh TH (2010) Composite edible films based on hydroxypropyl methylcellulose reinforced with microcrystalline cellulose nanoparticles. J Agric Food Chem 58(6):3753–3760

Biniecka M, Caroli S (2011) Analytical methods for the quantification of volatile aromatic compounds. TrAC Trends Anal Chem 30(11):1756–1770

Blandino A, Macias M, Cantero D (1999) Formation of calcium alginate gel capsules: influence of sodium alginate and $CaCl_2$ concentration on gelation kinetics. J Biosci Bioeng 88(6):686–689

Bodmeier R, Wang J (1993) Microencapsulation of drugs with aqueous colloidal polymer dispersions. J Pharm Sci 82(2):191–194

Borgogna M, Bellich B, Cesàro A (2011) Marine polysaccharides in microencapsulation and application to aquaculture: "from sea to sea". Mar Drugs 9(12):2572–2604

Borgogna M, Bellich B, Zorzin L, Lapasin R, Cesàro A (2010) Food microencapsulation of bioactive compounds: rheological and thermal characterisation of non-conventional gelling system. Food Chem

Bourtoom T (2008) Edible films and coatings: characteristics and properties. Int Food Res J 15 (3):237

Cayot N, Dury-Brun C, Karbowiak T, Savary G, Voilley a (2008) Measurement of transport phenomena of volatile compounds: a review. Food Res Int 41(4):349–362

Chai Y, Mei L-H, Wu G-L, Lin D-Q, Yao S-J (2004) Gelation conditions and transport properties of hollow calcium alginate capsules. Biotechnol Bioeng 87(2):228–233

Champagne CP, Fustier P (2007) Microencapsulation for the improved delivery of bioactive compounds into foods. Curr Opin Biotechnol 18:184–190

Chan AW, Neufeld RJ (2010) Tuneable semi-synthetic network alginate for absorptive encapsulation and controlled release of protein therapeutics. Biomaterials 31(34):9040–9047

Chen H, Ouyang W, Lawuyi B, Prakash S (2006) Genipin cross-linked alginate-chitosan microcapsules: membrane characterization and optimization of cross-linking reaction. Biomacromolecules 7(7):2091–2098

Chen L, Subirade M (2006) Alginate-whey protein granular microspheres as oral delivery vehicles for bioactive compounds. Biomaterials 27(26):4646–4654

Cook MT, Tzortzis G, Charalampopoulos D, Khutoryanskiy VV (2012) Microencapsulation of probiotics for gastrointestinal delivery. J Controlled Release 162(1):56–67

Coviello T, Matricardi P, Marianecci C, Alhaique F (2007) Polysaccharide hydrogels for modified release formulations. J Controlled Release 119:5–24

Da Silva MA, Bierhalz ACK, Kieckbusch TG (2009) Alginate and pectin composite films crosslinked with Ca^{2+} ions: effect of the plasticizer concentration. Carbohydr Polym 77 (4):736–742

De Moura MR, Aouada Fa, Avena-Bustillos RJ, McHugh TH, Krochta JM, Mattoso LHC (2009) Improved barrier and mechanical properties of novel hydroxypropyl methylcellulose edible films with chitosan/tripolyphosphate nanoparticles. J Food Eng 92(4):448–453

De Vos P, Faas MM, Spasojevic M, Sikkema J (2010) Encapsulation for preservation of functionality and targeted delivery of bioactive food components. Int Dairy J 20(4):292–302. Retrieved from <Go to ISI>://000275924800013

Desobry S, Netto FM, Labuza TP (1999) Influence of maltodextrin systems at an equivalent 25DE on encapsulated b-carotene loss during storage. J Food Process Preserv 23(1):39–55

Dogan N, McHugh TH (2007) Effects of microcrystalline cellulose on functional properties of hydroxy propyl methyl cellulose microcomposite films. J Food Sci 72(1):E016–E022

Dong Z, Wang Q, Du Y (2006) Alginate/gelatin blend films and their properties for drug controlled release. J Membr Sci 280(1–2):37–44

Dressman JB, Amidon GL, Reppas C, Shah VP (1998) Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. Pharm Res 15:11–22

Drusch S, Mannino S (2009) Patent-based review on industrial approaches for the microencapsulation of oils rich in polyunsaturated fatty acids. Trends Food Sci Technol 20:237–244

Drusch S, Regier M, Bruhn M (2012) Recent advances in the microencapsulation of oils high in polyunsaturated fatty acids. In McElhatton A, do Amaral Sobral PJ (eds) Novel technologies in food science—their impact on products, consumer trends and environment. Springer, New York, pp 159–181

Drusch S, Serfert Y, Scampicchio M, Schmidt-Hansberg B, Schwarz K (2007) Impact of physicochemical characteristics on the oxidative stability of fish oil microencapsulated by spray-drying. J Agric Food Chem 55:11044–11051

Drusch S, Rätzke K, Shaikh MQ, Serfert Y, Steckel H, Scampicchio M, Voigt I et al (2009) Differences in free volume elements of the carrier matrix affect the stability of microencapsulated lipophilic food ingredients. Food Biophys 4(1):42–48

Drusch S, Serfert Y, Van Den Heuvel A, Schwarz K (2006) Physicochemical characterization and oxidative stability of fish oil encapsulated in an amorphous matrix containing trehalose. Food Res Int 39(7):807–815

Edlund U, Yu Y, Zhu Ryberg Y, Krause-Rehberg R, Albertsson A-C (2012) Positron lifetime reveals the nano level packing in complex polysaccharide-rich hydrolysate matrixes. Anal Chem 84:3676–3681

Esser E, Tessmar JKV (2013) Preparation of well-defined calcium cross-linked alginate films for the prevention of surgical adhesions. J Biomed Mater Res B Appl Biomater 101(5):826–839

Falco GM, Poortinga AT, Oversteegen SM (2013) Transport of nitrogen gas in glassy maltodextrins. J Membr Sci 428(1):480–488

Falguera V, Quintero JP, Jiménez A, Muñoz JA, Ibarz A (2011) Edible films and coatings: structures, active functions and trends in their use. Trends Food Sci Technol 22(6):292–303

Faroongsarng D, Sukonrat P (2008) Thermal behavior of water in the selected starch- and cellulose-based polymeric hydrogels. Int J Pharm 352(1–2):152–158

Ferreiro M, Tillman L (2002) Alginate/poly-L-lysine microparticles for the intestinal delivery of antisense oligonucleotides. Pharm Res 19(6):755–764

Frankel EN (2005) Lipid oxidation. The Oily Press, Bridgwater

Galia E, Nicolaides E, Hörter D, Löbenberg R, Reppas C, Dressman JB (1998) Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. Pharm Res 15:698–705

George M, Abraham TE (2006) Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan-a review. J Controlled Release 114(1):1–14

Gianfrancesco A, Vuataz G, Mesnier X, Meunier V (2012) New methods to assess water diffusion in amorphous matrices during storage and drying. Food Chem 132(4):1664–1670

Gombotz WR, Wee SF (2012) Protein release from alginate matrices. Adv Drug Deliv Rev 64:194–205

Gouin S (2004) Microencapsulation: industrial appraisal of existing technologies and trends. Trends Food Sci Technol 15(7–8):330–347

Gåserød O, Sannes A, Skjåk-Bræk G (1999) Microcapsules of alginate–chitosan. II. A study of capsule stability and permeability. Biomaterials 20:773–783

Gåserød O, Smidsrød O, Skjåk-Bræk G (1998) Microcapsules of alginate-chitosan–I: a quantitative study of the interaction between alginate and chitosan. Biomaterials 19:1815–1825

Habig-McHugh T, Avena-Bustillos R, Krochta JM (1993) Hydrophilic edible films: modified procedure for water vapor permeability and explanation of thickness effects. J Food Sci 58 (4):899–903

Hambleton A, Perpiñan-Saiz N, Fabra MJ, Voilley A, Debeaufort F (2012) The Schroeder paradox or how the state of water affects the moisture transfer through edible films. Food Chem 132 (4):1671–1678

Hansen LT, Allan-Wojtas PM, Jin YL, Paulson AT (2002) Survival of Ca-alginate microencapsulated Bifidobacterium spp. in milk and simulated gastrointestinal conditions. Food Microbiol 19:35–45

Henning S, Leick S, Kott M, Rehage H, Suter D (2012) Sealing liquid-filled pectinate capsules with a shellac coating. J Microencapsul 29(2):147–155

Hoffman AS (2012) Hydrogels for biomedical applications. Adv Drug Deliv Rev 64:18–23

Hogan SA, O'Riordan ED, O'Sullivan M (2003) Microencapsulation and oxidative stability of spray-dried fish oil emulsions. J Microencapsul 20(5):675–688

Jackson LS, Lee K (1991) Microencapsulation and the food industry. Lebensmittel-Wissenschaft und Technolgie 24:289–297

Jacobsen C (1999) Sensory impact of lipid oxidation in complex food systems. Fett/Lipid 101:484–492

James B (2009) Advances in "wet" electron microscopy techniques and their application to the study of food structure. Trends Food Sci Technol 20(3–4):114–124

Jeleń HH, Majcher M, Dziadas M (2012) Microextraction techniques in the analysis of food flavor compounds: a review. Anal Chim Acta 738:13–26

Kailasapathy K (2002) Microencapsulation of probiotic bacteria: technology and potential applications. Curr Issues Intestinal Microbiol 3:39–48

Karewicz A, Zasada K, Szczubiałka K, Zapotoczny S, Lach R, Nowakowska M (2010) "Smart" alginate-hydroxypropylcellulose microbeads for controlled release of heparin. Int J Pharm 385 (1–2):163–169

Kilburn D, Claude J, Schweizer T, Alam A, Ubbink J (2005) Carbohydrate polymers in amorphous states: an integrated thermodynamic and nanostructural investigation. Biomacromolecules 6:864–879

Kilcast D, Subramaniam P (2000) The stability and shelf-life of food. Woodhead Publishing, Boca Raton

Kim W-T, Chung H, Shin I-S, Yam KL, Chung D (2008) Characterization of calcium alginate and chitosan-treated calcium alginate gel beads entrapping allyl isothiocyanate. Carbohydr Polym. Retrieved from http://www.sciencedirect.com/science/article/B6TFD-4P5NX4C-2/2/6280d9fc7ad3e18f6eddf71c4fd2b684

Kimura K, Nishimura H, Iwata I, Mizutani J (1983) Deterioration mechanism of lemon flavor. 2. Formation mechanism of off-odor. J Agric Food Chem 31(2):801–804

King CJ (1995) Retention of volatile compounds revisited. Drying Technol 13(5–7):1221–1240

Kosaraju SL (2005) Colon targeted delivery systems: review of polysaccharides for encapsulation and delivery. Crit Rev Food Sci Nutr 45(4):251–258
Koyama K, Seki M (2004) Evaluation of mass-transfer characteristics in alginate-membrane liquid-core capsules prepared using polyethylene glycol. J Biosci Bioeng 98(2):114–121
Kuang SS, Oliveira JC, Crean AM (2010) Microencapsulation as a tool for incorporating bioactive ingredients into food. Crit Rev Food Sci Nutr 50(10):951–968
Kuo CK, Ma PX (2001) Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: part 1. Structure, gelation rate and mechanical properties. Biomaterials 22(6):511–521
Lee DW, Hwang SJ, Park JB, Park HJ (2003) Preparation and release characteristics of polymer-coated and blended alginate microspheres. J Microencapsul 20(2):179–192
Leick S, Kemper A, Rehage H (2011a) Alginate/poly-L-lysine capsules: mechanical properties and drug release characteristics. Soft Matter 7(14):6684
Leick S, Kott M, Degen P, Henning S, Päsler T, Suter D, Rehage H (2011b) Mechanical properties of liquid-filled shellac composite capsules. Phys Chem Chem Phys 13(7):2765–2773
Lesmes U, Cohen SH, Shener Y, Shimoni E (2009) Effects of long chain fatty acid unsaturation on the structure and controlled release properties of amylose complexes. Food Hydrocolloids 23 (3):667–675. Retrieved from http://www.sciencedirect.com/science/article/B6VP9-4SK6310-6/2/b152813de05e1cc4871a10ed6967c6f9
Li XY, Chen XG, Cha DS, Park HJ, Liu CS (2009) Microencapsulation of a probiotic bacteria with alginate-gelatin and its properties. J Microencapsul 26(4):315–324
Liu P, Krishnan TR (1999) Alginate-pectin-poly-L-lysine particulate as a potential controlled release formulation. J Pharm Pharmacol 51(2):141–149
Lorén N, Langton M, Hermansson A-M (2007) Confocal fluorescence microscopy (CLSM) for food structure characterisation. In McClements DJ (ed) Understanding and controlling the microstructure of complex foods. CRC Press, Boca Raton, pp 232–260
López-Córdoba A, Deladino L, Martino M (2014) Release of yerba mate antioxidants from corn starch-alginate capsules as affected by structure. Carbohydr Polym 99:150–157
Mackie A (2012) Interaction of food ingredient and nutraceutical delivery systems with the human gastrointestinal tract. In: Garti N, McClements DJ (eds) Encapsulation technologies and delivery systems for food ingredients and nutraceuticals. Woodhead Publishing, Oxford, pp 49–70
Madene A, Jacquot M, Scher J, Desobry S (2006) Flavour encapsulation and controlled release—a review. Int J Food Sci Technol 41:1–21
Mahou R, Wandrey C (2010) Alginate–poly(ethylene glycol) hybrid microspheres with adjustable physical properties. Macromolecules 43(3):1371–1378
Maiani G, Castón MJP, Catasta G, Toti E, Cambrodón IG, Bysted A, Granado-Lorencio F et al (2009) Carotenoids: actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. Mol Nutr Food Res 53(2):S194–S218
Marques MRC, Loebenberg R, Almukainzi M (2011) Simulated biological fluids with possible application on dissolution testing. Dissolution Technol 18:15–28
Martinsen A, Skjåk-Braek G, Smidsrød O (1989) Alginate as immobilization material: I. Correlation between chemical and physical properties of alginate gel beads. Biotechnol Bioeng 33(1):79–89
Martinsen A, Skjåk-Bræk G, Smidsrød O (1991) Comparison of different methods for determination of molecular weight and molecular weight distribution of alginates. Carbohydr Polym 15:171–193
Meares P (1954) The diffusion of gases through polyvinyl acetate. J Am Chem Soc 76:3415–3422
Morris VJ (2007) Atomic force microscopy (AFM) techniques for characterising food structure. In: McClements DJ (ed) Understanding and controlling the microstructure of complex foods. CRC Press, Boca Raton, pp 209–231
Murano E (1998) Use of natural polysaccharides in the microencapsulation techniques. J Appl Ichthyol 14(3–4):245–249

Murata Y, Nakada K, Miyamoto E (1993) Influence of erosion of calcium-induced alginate gel matrix on the release of brilliant blue. J Controlled Release 23(1):21–26
Nguyen H, Campi EM, Roy Jackson W, Patti AF (2009) Effect of oxidative deterioration on flavour and aroma components of lemon oil. Food Chem 112(2):388–393
Onwulata CI (2012) Encapsulation of new active ingredients. Ann Rev Food Sci Technol 3:183–202
Prüße U, Dalluhn J, Breford J, Vorlop K-D (2000) Herstellung sphärischer Partikel mit dem Strahlschneider-Verfahren. Chemie Ingenieur 72:852–858
Qussi B, Suess W (2005) Investigation of the effect of various shellac coating compositions containing different water-soluble polymers on In vitro drug release. Drug Dev Ind Pharm 31 (1):99–108
Reineccius G (2010) Flavor deterioration during food storage. In: Skibsted LH, Risbo J, Andersen ML (eds) Chemical deterioration and physical instability of food and beverages. Woodhead Publishing, Oxford, pp 95–112
Rhim J-W, Ng PKW (2007) Natural biopolymer-based nanocomposite films for packaging applications. Crit Rev Food Sci Nutr 47(4):411–433
Ridgway K, Lalljie S, Smith R (2010) Analysis of food taints and off-flavours: a review. Food Addit Contam A Chem Anal Control Exposure Risk Assess 27:146–168
Rinaudo M (2008) Main properties and current applications of some polysaccharides as biomaterials. Polym Int 57:397–430
Roopa BS, Bhattacharya S (2008) Alginate gels: I. Characterization of textural attributes. J Food Eng 85(1):123–131
Ross CF (2009) Sensory science at the human–machine interface. Trends Food Sci Technol 20 (2):63–72
Roos YH (2010) Glass transition temperature and its relevance in food processing. Ann Rev Food Sci Technol 1:469–496
Rulkens WH, Thijssen HAC (1972) The retention of organic volatiles in spray-drying aqueous carbohydrate solutions. J Food Technol 7:95–105
Sagalowicz L, Leser ME (2010) Delivery systems for liquid food products. Curr Opin Colloid Interface Sci 15:61–72
Sandoval-Castilla O, Lobato-Calleros C, García-Galindo HS, Alvarez-Ramírez J, Vernon-Carter EJ (2010) Textural properties of alginate–pectin beads and survivability of entrapped Lb. casei in simulated gastrointestinal conditions and in yoghurt. Food Res Int 43(1):111–117
Saravanan M, Rao KP (2010) Pectin–gelatin and alginate–gelatin complex coacervation for controlled drug delivery: influence of anionic polysaccharides and drugs being encapsulated on physicochemical properties of microcapsules. Carbohydr Polym 80(3):808–816
Schieberle P, Grosch W (1988) Identification of potent flavor compounds formed in an aqueous lemon oil/citric acid emulsion. J Agric Food Chem 36(4):797–800
Schoonman A, Ubbink J, Bisperink C, Meste L, Karel M (2002) Solubility and diffusion of nitrogen in maltodextrin/protein tablets. Biotechnol Prog 18:139–154
Schrooyen PMM, Van der Meer R, De Kruif CG (2001) Microencapsulation: its application in nutrition. Proc Nutr Soc 60:475–479
Serfert Y, Lamprecht C, Tan C-P, Appel E, Selhuber-Unkel C, Gorb S, Schwarz K et al (2013) Characterization and use of β-lactoglobulin fibrils for microencapsulation of lipophilic ingredients by spray drying. J Food Eng 143:53–61
Sezer A, Akbuga J (1999) Release characteristics of chitosan treated alginate beads: I. Sustained release of a macromolecular drug from chitosan treated alginate beads. J Microencapsul 16 (2):195–203
Singh B, Sharma DK, Gupta A (2009) A study towards release dynamics of thiram fungicide from starch-alginate beads to control environmental and health hazards. J Hazard Mater 161(1):208–216
Smidsrød O, Skjåk-Braek G (1990) Alginate as immobilization matrix for cells. Trends Biotechnol 8(3):71–78

Smidsrød O, Glover R, Whittington S (1973) The relative extension of alginates having different chemical composition. Carbohydr Res 27(1):107–118
Steele R (2000) Understanding and controlling the shelf-life of food. Woodhead Publishing, Boca Raton
Tan LH, Chan LW, Heng PWS (2009) Alginate/starch composites as wall material to achieve microencapsulation with high oil loading. J Microencapsul 26(3):263–271
Tanaka H, Matsumura M, Veliky IA (1984) Diffusion characteristics of substrates in Ca-alginate gel beads. Biotechnol Bioeng 26(1):53–58
Townrow S, Kilburn D, Alam A, Ubbink J (2007) Molecular packing in amorphous carbohydrate matrixes. J Phys Chem B 111:12643–12648
Townrow S, Roussenova M, Giardiello M-I, Alam A, Ubbink J (2010) Specific volume-hole volume correlations in amorphous carbohydrates: effect of temperature, molecular weight, and water content. J Phys ChemB 114:1568–1578
Tuorila H, Monteleone E (2009) Sensory food science in the changing society: opportunities, needs, and challenges. Trends Food Sci Technol 20(2):54–62
Turek C, Stintzing FC (2013) Stability of essential oils: a review. Compr Rev Food Sci Food Saf 12(1):40–53
Ubbink J (2016) Structural and thermodynamic aspects of plasticization and antiplasticization in glassy encapsulation and biostabilization matrices. Adv Drug Deliv Rev 100(1):10–26
Ubbink J, Krüger J (2006) Physical approaches for the delivery of active ingredients in foods. Trends Food Sci Technol 17(5):244–254
Ueno T, Kiyohara S, Ho C-T, Masuda H (2006) Potent inhibitory effects of black tea theaflavins on off-odor formation from citral. J Agric Food Chem 54(8):3055–3061
Uhlemann J, Reiß I (2010) Product design and process engineering using the example of flavors. Chem Eng Technol 33(2):199–212
Van Boekel MAJS (2009) Kinetic modeling of reactions in food. CRC Press, Boca Raton
Vega C (2006) Invited review: spray-dried dairy and dairy-like emulsions-compositional considerations. J Dairy Sci 89:383–401
Venkateshwarlu G, Let MB, Meyer AS, Jacobsen C (2004) Chemical and olfactometric characterization of volatile flavor compounds in a fish oil enriched milk emulsion. J Agric Food Chem 52:311–317
Wang S, Langrish T (2009) A review of process simulations and the use of additives in spray drying. Food Res Int 42(1):13–25
Wang W, Waterhouse GIN, Sun-Waterhouse D (2013) Co-extrusion encapsulation of canola oil with alginate: effect of quercetin addition to oil core and pectin addition to alginate shell on oil stability. Food Res Int 54(1):837–851
Whorton C, Reineccius GA (1995) Evaluation of the mechanisms associated with the release of encapsulated flavor materials from maltodextrin matrices. Encapsulation and controlled release of food ingredients. American Chemical Society, Washington
Wichchukit S, Oztop MH, McCarthy MJ, McCarthy KL (2013) Whey protein/alginate beads as carriers of a bioactive component. Food Hydrocolloids 33(1):66–73
Yampolskii Y, Shantarovich V (2006) Positron annihilation lifetime spectroscopy and other methods for free volume evaluation im polymers. In Yampolskii YP, Pinnau I, Freeman BD (eds) Materials science of membranes for gas and vapor separation. Wiley, New York, pp 191–210
Yampolskii YP, Pinnau I, Freeman BD (eds) (2006) Materials science of membranes for gas and vapor separation. Wiley VCH, Weinheim
Zabar S, Lesmes U, Katz I, Shimoni E, Bianco-Peled H (2010) Structural characterization of amylose-long chain fatty acid complexes produced via the acidification method. Food Hydrocolloids 24(4):347–357

Chapter 12
Food Extrusion

Zeki Berk

Abstract Food extrusion is an increasingly important and widely applied process in the industry, and at the same time is a subject of extensive scientific and engineering research activity. The complexity of the extrusion process is prompting research in areas such as fluid dynamics, rheology and chemical reactions. The introduction of the twin-screw co-rotating extruder with flexible screw configuration is a marked improvement in the process. In addition to its use as an efficient tool for the production of pasta, snacks, ready-to-eat (RTE) cereals, texturized proteins and animal feed, the extruder is a valuable high-temperature-short-time (HTST) reactor. Extruded food products are safe, nutritious and of high organoleptic quality. Progress in extrusion technology is expected to contribute to the global food supply.

Keywords Cooking extrusion · Single-screw extruder · Twin-screw extruder · Flow pattern · Mixing · Residence time distribution · High moisture extrusion · Co-extrusion · Reactive extrusion · Texturization · Pellets · Direct expansion · Cold extrusion · Nutritional aspects · Food safety · Food availability

12.1 Introduction

Food extrusion is an increasingly important and widely applied process in the industry, and at the same time is a subject of extensive scientific and engineering research activity. Industrially, extrusion is the central process in the production of pasta products, expanded and filled snacks and ready-to-eat (RTE) breakfast items, pre-cooked cereals and legumes, infant foods, pet foods, texturized meat analogs and numerous confectionery items including chocolate, edible films, modified starch, starch hydrolysates, ice cream and more (Frame 1994; Riaz 2000; Guy 2001). From a scientific perspective, the complex nature of the extrusion process has driven and continues to drive extensive research in areas such as fluid dynamics, rheology and chemical reactions.

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Literally, the word "extrusion" means the action of pushing out material through a narrow opening (from the Latin *extrūdere*, *ex* = out + *trudere* = push, thrust). This definition fits well with the operation of extrusion in processing metals, plastic polymers, most pasta products and animal feed pellets, whereby the principal role of the extruder is to impart to the product a certain shape, depending on the cross section of the opening and the cutting device, without otherwise affecting the physical and chemical properties of the extruded material. Without underestimating the importance of such applications of "forming extrusion", one can say that the type of extrusion that has had the most significant impact on the food industry is the thermomechanical process known as "extrusion cooking", whereby significant modifications in the chemical composition and physical properties of the extruded product are induced. In extrusion cooking, the combined action of mechanisms such as heat transfer (both heating and cooling), mass transfer, shear, compression and expansion results in effects such as cooking, thermal processing, stabilization, mixing, kneading, melting, cooling, freezing (ice cream), chemical reactions (protein denaturation, starch gelatinization, sugar caramelization), texturization, coating, puffing, kneading and forming, all in one operation.

Long before its application in foods, industrial extrusion was widely used in metallurgy and in the processing of plastic polymers. The first extrusion patent for making lead pipe was issued in 1797 (Kazemzadeh 2012). Production of pasta products by continuous extrusion started in the late 1930s (Mercier and Cantarelli 1986), and cooking extruders for making puffed snacks were developed in the 1940s (Harper 1989). In the 1960s, simple low-cost extruders were developed for on-farm cooking of soybeans, used as animal feed. A decade later, low-cost extruder-cooker (LEC) technology was adopted and promoted by international agencies and governments for the production of low-cost infant foods based on oilseed–cereal mixtures (Crowley 1979). Extrusion cooking has been used for the texturization of proteinaceous materials of vegetable origin to produce meat-like structures (Berk 2013), which continues to be one of the principal applications of extrusion cooking. Recently, extrusion has been applied to the development of biodegradable and nanocomposite films with interesting barrier characteristics (Kumar et al. 2010; Li et al. 2011).

The first food extruder-cookers were single-screw machines. Twin-screw extruders for foods were introduced in the 1970s, widening the scope of food extrusion cooking considerably.

The rapid expansion of cooking extrusion in the food processing industry may be explained in light of the specific advantages of this process over other processes serving the same purpose. Some of these specific advantages are listed below:

- Extrusion is a continuous, single-pass process.
- Extrusion is a multi-functional process. A number of effects may be achieved simultaneously in the same extruder.
- The same extruder, with certain modifications, may be used with different materials, for different purposes, resulting in a vast variety of different products.
- For its production capacity, the extruder is relatively compact and requires little plant space.

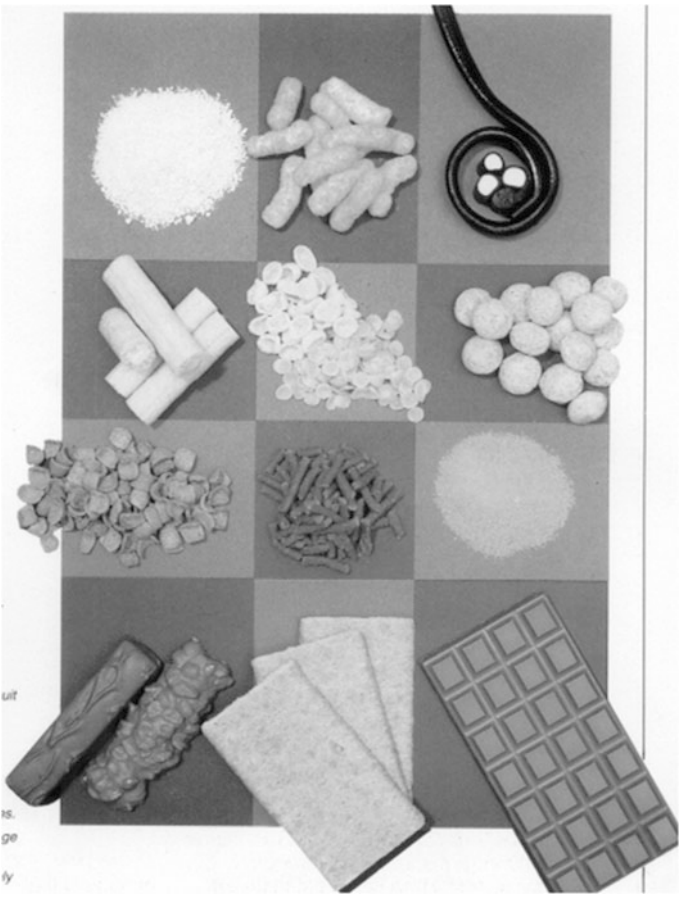

Fig. 12.1 Some extruded products (Courtesy of Baker Perkins Limited)

- Extrusion easily lends itself to automation and control. It requires little labor or supervision.
- The average retention time in extruders is usually short, and the retention time distribution is fairly narrow.

These advantages explain the rapid expansion of extrusion processes in the food industry. A limited array of foods produced by extrusion is presented in Fig. 12.1.

12.2 The Extruder

Harper (1978) describes the food extruder as consisting of "a flighted Archimedes screw which rotates in a tight fitting cylindrical barrel". While this description may have corresponded to the structure of some of the earliest single-screw extruders,

the present-day extruder is physically much more complex. The first use of single-screw extruders in food processing for continuous extrusion of pasta dates back to 1935 (Bruin et al. 1978).

12.2.1 The Single-Screw Extruder

(a) **Basic structure**

The single-screw extruder has a simple basic structure, consisting of the following components (Fig. 12.2):

- A hollow cylindrical shell, called the "barrel". The barrel can be either smooth or grooved. Extruders with conical barrels exist (Meuser and Wiedmann 1989), but they are not used in food processing.
- A flighted *screw* (also known as the "worm"), with a thick shaft (also known as the "screw root") and shallow flights, turning inside the barrel. The flights of the rotating screw convey the material along a helicoidal path (known as the "flow channel") formed between the flights, the screw root and the barrel. The width of the flow channel, resulting from the screw pitch, is considerably larger than its thickness, resulting from the distance between the root and the surface of the barrel. To maintain the positive displacement capacity of the extruder, the gap between the screw tip and the barrel surface is made as narrow as possible.
- At the feed end of the extruder, a feeding device such as a gravity hopper or an auger.
- At the exit end of the extruder, a restricted outlet, known as the die. The die serves as both a pressure release valve and a shape-forming element, imparting to the extrudate the desired shape, determined by the cross section of the aperture(s). The die is sometimes preceded by a perforated breaker plate, the function of which is to distribute the compressed material evenly across the die.
- A cutter for cutting the extrudate emerging from the die into pieces of known size. The cutter can simply be a blade rotating in a plane perpendicular to the exit direction.
- Additional elements for heating or cooling the barrel (steam or water jackets, electrical resistance heaters, induction heaters, etc.) These elements, external to the barrel, may be divided into individual segments in order to impose different temperature profiles at different sections of the extruder.

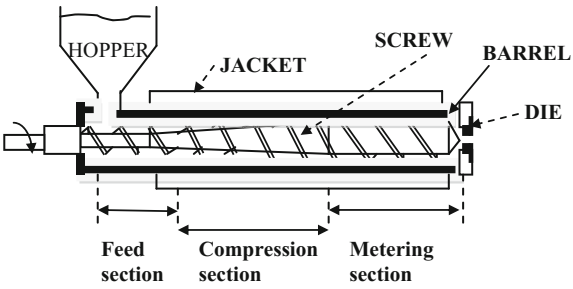

Fig. 12.2 Basic structure of a single-screw extruder

- At selected positions of the barrel, openings or ports for the introduction of steam, water and additional feed materials, as needed for the process.
- Ports for pressure release.
- Sensors for measuring and controlling temperature and pressure.
- A powerful motor and a drive, with speed and direction variation capability and appropriate protection devices.

(b) Operation

The feed materials processed in cooker-extruders are typically particulate solids (flours, powders, meals, etc.) at a certain water content. Prior to extrusion, the feed material may be subjected to "conditioning" operations such as water equilibration, pre-mixing or pre-heating. The feed is then introduced into the machine through the hopper by gravity or by an auger. Additional water or other fluids may be fed directly to the extruder through appropriate ports. As the screw rotates, the flights drag the material forward towards the die end of the extruder. Friction with the moving material occurs on both the screw and barrel surfaces. Preferably, friction with the barrel surface is stronger than friction with the screw. In the opposite case, occurring if the water content of the material is high, the melt sticks to the screw and turns with it without advancing. This undesirable effect is known as "cylindering", and is less likely to occur in extruders with slotted barrels. Due to friction, a large part of the mechanical energy is internally converted to heat, and the temperature of the material rises significantly. Additional heat may be supplied by externally heated barrels. As a result of shear and high temperature, the particulate structure of the material disappears, and the mass is converted to a viscous dough. Similar to that produced in the extrusion of plastic polymers, this viscous mass is called "melt". At the same time, chemical reactions such as protein denaturation, starch gelatinization and partial hydrolysis take place (Camire 1998). The kinetics of these reactions was reviewed by Zhao et al. (2011).

The screw and barrel are constructed in such a way as to progressively reduce the cross section of the flow channels. As a result, the material is increasingly compressed as it moves down the extruder. The "compression ratio" of a screw is defined as the ratio of the cross-sectional area of the flow channel at the feed end to that at the exit end. Compression can be achieved by several types of screw configurations. The most

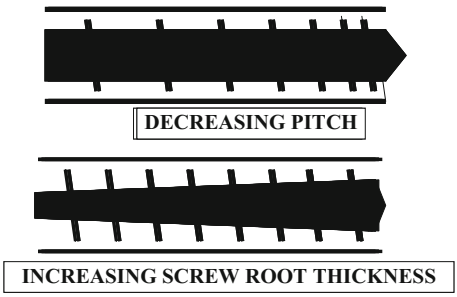

Fig. 12.3 Two methods of increasing compression ratio

common methods of compression call for either progressively decreasing screw pitch, progressively increasing root thickness, or both (Fig. 12.3). Screw configurations resulting in compression ratios between 1.5 and 4 are the most common.

In most single-screw extruders, screw configuration is not uniform over the entire length of the machine. The extruder can typically be divided into three sections, each with different screw structures (Fig. 12.2), as follows:

- Feed section: In this section, the screw pitch and root diameter are constant. The material coming from the feed hopper is simply conveyed forward, as in a screw conveyor, almost without compression or texture modification.
- Transition section (compression section): In this section, the mass is heated and compressed.
- Metering section: Here, the cross-sectional area of the flow channel is nearly constant. Therefore, there is almost no compression, but most of the effects of extrusion (melting, texturization, kneading, chemical reactions, etc.) occur in this section as a result of extensive shearing and mixing. At the end of the metering section, the melt is delivered to the die assembly.

As mentioned above, most of the mechanical energy used for turning the screw is dissipated into the material as heat. This is heat generated in situ and not supplied through heat transfer. Additional heat may be supplied by the externally heated barrel surface or by direct injection of live steam. In single-screw extruders, the heat generated by viscous dissipation constitutes the major portion of the energy input. Consequently, heating in a single-screw cooking extruder is extremely rapid.

Instantaneous puffing at the exit from the die is another important effect of extrusion cooking. As a result of compression, the melt may reach pressure of up to 20 MPa (Bruin et al. 1978). At such high pressure, the moist melt does not boil, despite its moisture content and temperatures as high as 180–200 °C. However, as the pressure is suddenly released into the atmosphere at the exit from the die, some of the water in the product is instantaneously evaporated, and as a result the product is puffed. Air bubbles inevitably entrapped in the melt through mixing in the extruder also contribute to puffing (Cisneros and Kokini 2002). An interesting alternative to puffing by flash evaporation of water makes use of supercritical fluid carbon dioxide. Supercritical CO_2 is injected into the melt in the extruder and penetrates the melt by diffusion. As the pressure drops at the exit from the die, gaseous CO_2 is desorbed and puffing occurs. Puffing by this method is reported to be less explosive, resulting in a product with improved porous structure, smooth surface and light color (Ferdinand et al. 1990; Mulvaney and Rizvi 1993; Ayoub and Rizvi 2011; Sauceau et al. 2011; Wang and Ryu 2013).

The extent of puffing can be controlled by releasing some of the pressure through an appropriate port at the metering section and/or by lowering the temperature of the melt just before the die.

Energy consumption in extrusion is an important technological and commercial factor. It is usually expressed as “specific mechanical energy (SME)”, which is the actual net mechanical energy invested per unit mass of product. The net mechanical

energy input is calculated by multiplying the torque by the angular speed of the screw and dividing the result by the mass flow rate of the extruder.

(c) Flow patterns, extruder throughput

Since the development of the extruder, numerous efforts have been devoted to the study of the flow patterns inside the machine (Carley et al. 1953; Pinto and Tadmor 1970; Bigg and Middelman 1974; Bruin et al. 1978; Tadmor and Gogos 1979; Harper 1980; Bounié 1988; Tayeb et al. 1992). Most of these studies have dealt with extruders for processing plastic polymers in plasticators, and later applied with varying degrees of success to cooking extruders for food materials by simulation. The interest in studying flow inside the extruder stems from the need to understand the mechanisms of mixing and residence time distribution (RTD) and to develop viable theories for the prediction of material throughput rates, pressure drop and power consumption (Bruin et al. 1978). Numerous mechanical models have been proposed for analysis of the complex flow patterns in the extruder. In the most common early models, the helicoidal flow channel is mentally "peeled off" the screw and laid flat (Harper 1980), so as to have a straight flow channel of rectangular cross section. Most commonly, only the metering section, where there is no compression and no acceleration due to a gradual decrease in the cross section, is considered. For convenience, the screw is considered static and movement is attributed to the barrel. In most studies, it is assumed that the melt is a Newtonian fluid.

The movement of the melt inside the flow channel is defined by the components of its velocity in the direction of the axes x, y and z, corresponding to the width, depth and length of the channel, respectively. Flow in the x direction contributes to mixing, and flow in the y direction is usually negligible. Net movement in the z direction (direction of the extruder axis) determines the material flow rate. Two different flow elements occur in the z direction. The material is pushed forward by the turning flights. This is "drag flow", and it depends on the velocity component in the z direction and the cross section of the flow channel. The velocity in the z direction, v_z, depends on the screw diameter D, rotation speed N and lead angle of the screw θ.

$$v_z = \pi DN \cos\theta$$

It follows that the drag flow rate F_{drag} is given by:

$$F_{\text{drag}} = \pi DN \cos\theta \, WH/2$$

where W and H are the width and height of the channel. The drag flow rate represents the positive displacement capability of the extruder.

The second flow element in the longitudinal direction is the "backflow" or "pressure flow". As a result of gradual compression, a pressure gradient is created in a direction opposite that of the drag. The effect of this gradient dP/dz is the flow element F_{pressure} given by:

$$F_{\text{pressure}} = \left[\frac{WH^3}{12\mu}\left(\frac{dP}{dz}\right)\right]$$

where μ is the apparent viscosity of the melt, assuming Newtonian rheology.

The pressure flow rate can be regarded as the deviation of the extruder from true positive displacement pump behavior. The ratio of pressure flow to drag flow is a parameter of extruder performance (Harper 1980). Subtracting the pressure flow from the drag flow, one obtains the net volumetric throughput Q of the extruder, subject to the simplifying assumption made:

$$Q = \left[\frac{(\pi DN\ \cos\theta)WH}{2}\right] - \left[\frac{WH^3}{12\mu}\left(\frac{P_2 - P_1}{L}\right)\right]$$

where P_1 and P_2 are the pressure at the feed end and exit end, respectively, and L is the length of the extruder.

The above development is only approximate and cannot serve for design purposes because of the complex nature of flow in the extruder, and even more so because of the complex rheology of the food materials extruded. Model systems assuming non-Newtonian (e.g. power law) rheology, more suitable for polymer melts but not food doughs, have also been studied. Bruin et al. (1978) carried out a detailed experimental study with biopolymers (corn grits, soybean flour, modified amylopectin). At the conclusion of their extensive study, the authors wrote: "The prediction of flow rates during extrusion of biopolymers in single screw extruders is at present not well possible. An important reason is the lack of rheological data for materials under extrusion conditions. A second important reason is that most biopolymers undergo chemical reactions during extrusion cooking." However, the following qualitative approximate relationships may be concluded from the above analysis:

- Extruder output increases with screw diameter, speed of rotation, flow channel cross section and the area of die aperture.
- The backflow is strongly influenced by melt viscosity. Lower viscosity (e.g. due to higher moisture content) results in an increase in backflow and a corresponding decrease in net throughput.

(d) **Mixing**

One of the important functions of the extruder is mixing, with the objective of increasing the homogeneity of the contents. The basic mechanism of mixing consists in moving parts of the material in relation to each other. In the single-screw extruder, the movement of parts of the fluid in different directions occurs mainly because of the existence of the pressure flow (back flow), and to a lesser extent because of the transversal flow (in the x direction) within the flow channel (Fig. 12.4). Consequently, any factor capable of reducing the backflow (higher melt

viscosity, smaller longitudinal pressure gradient, larger die opening) may be expected to diminish mixing efficiency.

Twin-screw extruders are better "mixers" than their single-screw counterparts due to specific screw configurations, to be described later.

(e) **Residence time and residence time distribution**

Important chemical reactions, such as gelatinization of starch, hydrolysis of carbohydrates and peptides, denaturation of proteins and Maillard reactions, take place during extrusion cooking. One branch of extrusion studies, reactive extrusion, deals specifically with extrusion-driven chemical reactions (Kokini 1993; Manoi and Rizvi 2009; Steinmacher et al. 2012; deMesa-Stonestreet et al. 2012). At any rate, the extruder can be viewed as a reactor, and as with any reactor, residence time (RT) and—even more so—RTD become important process parameters. RT and RTD refer primarily to the length of time during which the processed material or portions of it are subjected to a certain treatment (Bimbenet et al. 2002). The pathway of every particle of material in a continuous reactor is too complex for analytical treatment. Therefore, simplified models of reactor behavior have been developed (Berk 2013). Two of these models (Fig. 12.5) and their variations have been proposed for predicting RT and RTD in extruders:

- The plug flow reactor (PFR): In this model, as its name indicates, the material moves as a block or plug. Flow velocity is uniform throughout, and there is no mixing within the fluid. Therefore, the RT is the same for every portion of the fluid. The RTD curve is flat.
- The continuous stirred tank reactor (CSTR): This type of reactor simulates a perfectly agitated tank or vessel, continuously fed and discharged. The assumption of perfect mixing dictates that the composition and all other conditions at a given time are uniform at all points within the reactor. The

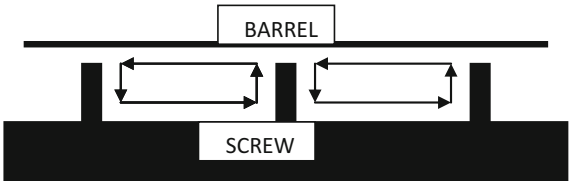

Fig. 12.4 Mixing by cross-flow

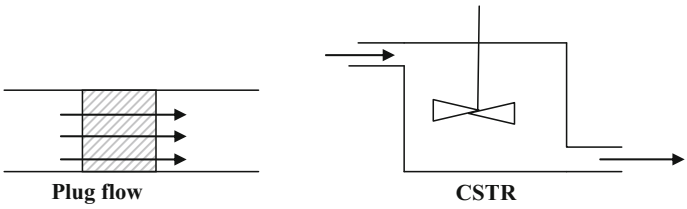

Fig. 12.5 Two reactor models

composition of the fluid discharged is the same as that of the bulk material in the reactor at the same instant.

Real reactors do not behave like the idealized models. RT is usually not the same for each portion of the material, hence the need to consider the RTD. In cooking extrusion, a narrow RTD is obviously preferred. RTD can be evaluated experimentally and modeled using statistical functions and parameters as described below.

- The RDT function $E(t)$, known as the frequency density function, refers to the probability of a given particle to reside in the reactor for a time equal to t. Figure 12.6a shows a hypothetical plot of the $E(t)$ function versus t. The area of the shaded strip in this plot represents the mass fraction of the fluid that has spent a time between t and $t + \Delta t$ in the reactor.
- The RDT function $F(t)$, referred to as the cumulative distribution function, represents the mass fraction of the material that has spent time t or less in the reactor. Figure 12.6b shows a hypothetical plot of the $F(t)$ function versus t.

The mean RT t_m is:

$$t_m = \int_0^\infty t \,.\, E(t) \,.\, dt$$

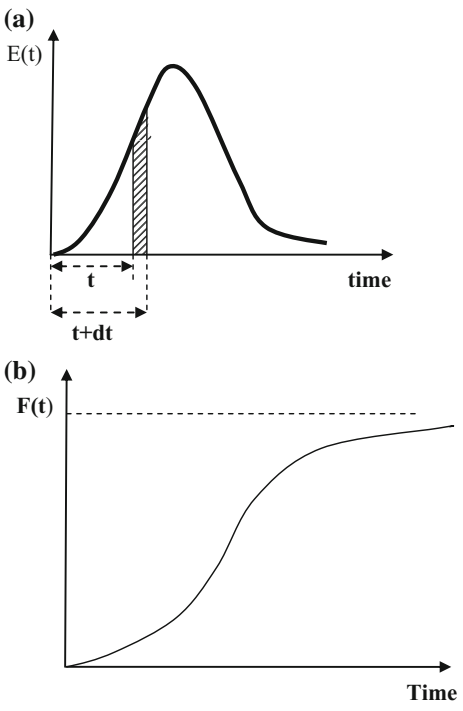

Fig. 12.6 **a** Hypothetical plot of E_t versus t. **b** Hypothetical plot of F_t versus t

The mean RT is equal to the mean travel time through the reactor (space time), τ, given approximately by:

$$t_m \approx \tau = \frac{V}{Q}$$

where V is the active volume of the reactor (the extruder, in our case) and Q is the volumetric flow rate.

RTD can be evaluated experimentally, most commonly by the *pulse injection* method (Fig. 12.7). At time 0, a small quantity of a tracer, such as a radioactive substance, is fed into the reactor. The concentration of the tracer (e.g. radioactivity) at the exit from the reactor is measured and recorded as a function of time. If C is the concentration of the tracer at the exit, $E(t)$ is given by:

$$E(t) = \frac{C}{\int_0^\infty C\mathrm{d}t}$$

A sharp peak with little tailing on either side indicates uniform RTD.

RTD in single-screw extruders has been studied extensively (Pinto and Tadmor 1970; van Zuilichem et al. 1973; Bigg and Middleman 1974; Bruin et al. 1978), both theoretically (according to velocity profiles) and experimentally. Relatively good fit between the predictions and the experimental results have been observed, particularly when working with Newtonian fluids. In general, RT is shortened by increasing the feed rate or the screw speed. Increasing the screw speed widens the RTD. Increasing the pressure flow causes the extruder to behave more like a PFR (Bruin et al. 1978). Working with rice flour, Yeh and Jaw (1998) found that a model consisting of a PFR in series with a CSTR fit well with the experimental data.

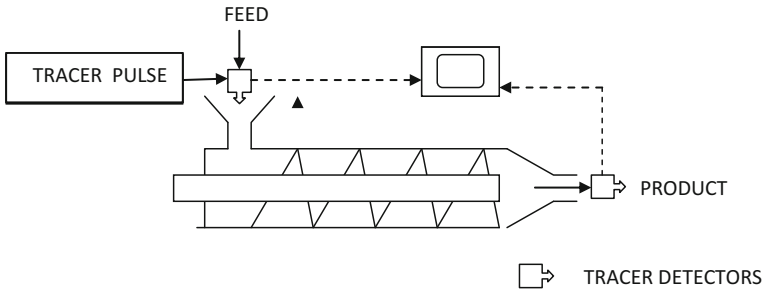

Fig. 12.7 Basic setup for the pulse injection method

12.2.2 The Twin-Screw Extruder

Suggestions to add a second screw to the extruder, with the objective of overcoming the problem of adhesion of highly viscous materials to the screw shaft (self-cleaning), were made already at the end of the nineteenth century (Ullrich 2008). Many patents were accorded, but the improvement of the concept, the practical application and the building of industrial machines was a lengthy process, and twin-screw extruders became available to the chemical and polymer industry only in the early 1940s. Their introduction to the food industry for extrusion cooking dates from the mid-1970s (Yacu 1985).

The twin-screw cooking extruder is not just a single-screw machine to which a second screw has been added, but a totally different machine in structure, operation, behavior and results. Harper (1992) lists the comparative advantages of the twin-screw extruder over its single-screw counterpart:

- Pumping efficiency is better and less dependent on the flow properties (viscosity, stickiness) of the processed material.
- Mixing is more complete.
- The rate of heat exchange rate from the barrel surface to the material is faster and more uniform.
- RTD is more uniform.
- The possibility of flexible modular configuration of the screw and the barrel makes the twin-screw extruder a much more versatile machine.
- High-moisture and sticky materials can be handled. This is probably the most important advantage, because it enables processing of materials that could not be handled previously by single-screw extruders. The introduction of twin-screw technology has resulted in the most significant expansion of the application of extrusion to foods and the spectrum of extruded products.
- Self-wiping reduces the risk of residue buildup.
- Feeding problems with cohesive materials are less serious.

Because of these and other advantages, the use of twin-screw extruders in the food industry grew rapidly, at the expense of the single-screw extruder. On the other hand, for equal output, a twin-screw extruder is considerably more expensive than a single-screw machine. The operating and maintenance costs are also higher. The mechanical complexity of the twin-screw extruder makes it less robust and more sensitive to abuse, and it requires more skilled supervision.

(a) **Basic structure**

Twin-screw extruders feature a pair of parallel screws rotating inside an enclosure or barrel with a figure-8-shaped cross section. At the entrance end there is a feeding device such as a gravity hopper or an auger-type conveyor. The hopper may be vibrated if the feed material is not free-flowing. At the exit end, each half of the barrel converges into a short conical section, each with a die at the apex (Fig. 12.8).

Alternatively, the barrel may converge into a single conical section ending in a single common die. Multi-die extruders are also available.

The screws can be co-rotating or counter-rotating (Fig. 12.9), closely intermeshing or distant. The co-rotating, closely intermeshing (self-wiping) extruders constitute the type most commonly employed in the food industry (Harper 1989). In the following discussion, we shall refer only to this type of extruder.

One of the important advances that accompanied the twin-screw extruder was the "tailor-made" screw, imparting to the extruder a degree of versatility not available to the older fixed-screw machines. The screws can be assembled by the user according to the desired configuration by sliding different screw elements on splinted shafts (Fig. 12.10). The short screw elements supplied by the manufacturer comprise leading, restricting, mixing, kneading, reverse-flow and many other types

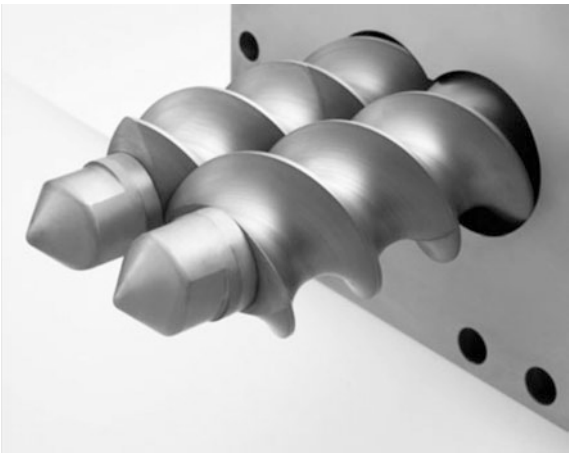

Fig. 12.8 Die end of twin screws (Courtesy of Baker Perkins Limited)

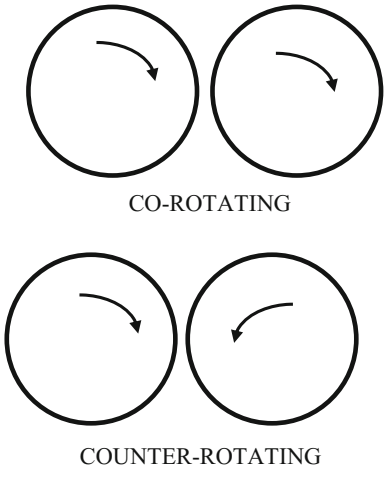

Fig. 12.9 Rotation directions in twin-screw extruder

Fig. 12.10 Twin screws for flexible configuration

Fig. 12.11 Screw elements for assembly on splinted shaft

of pieces with different pitch lengths (Fig. 12.11). The barrels are also different. In some models, the barrel is split into two halves longitudinally (clamshell barrel), allowing easy opening for removing and changing screws, inspection and cleaning. In other models (modular barrel), the barrel consists of short, separable sections or modules (Fig. 12.12).

(b) **Operation**

In the feed section, the material is rapidly conveyed forward by the two rotating screws. Typically, conveying screw elements with large lead angles are installed on the screw shaft in this section. As twin-screw extruders are usually starve-fed (Yacu 1985) and rapidly evacuated, the feed section is only partially full. The feeding section acts essentially as a fairly long heated screw conveyor with two screws. Due to the low holdup, the material is rapidly heated almost up to the temperature of the barrel surface. If needed, a short length of mixing elements can be included in the

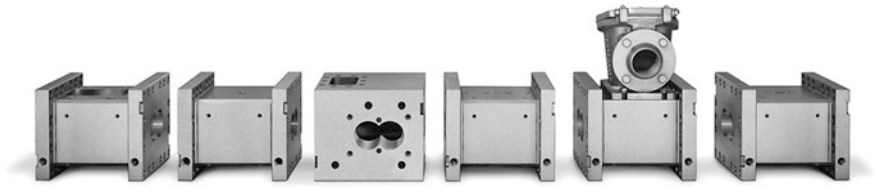

Fig. 12.12 Modular barrel

screw configuration at the end of the section, with the objective of giving the feed additional mixing.

Further down the barrel, in the melting and metering section, flow-restricting screw elements such as reverse-pitch units and barrel valves provide compression, promote heating and melting, and increase the filling ratio. The filling ratio in this section increases gradually towards the die.

Due to the dragging action of the second screw, friction is less than in single-screw extruders. Therefore, the proportion of mechanical energy converted to heat is lower, and heating depends primarily on heat transfer from the externally heated barrel. Most of the mechanical power is used for conveying. Cylindering is less likely to occur even with high-moisture feeds. Consequently, the positive displacement capability of the twin-screw conveyor is considerably higher.

(c) Flow patterns, mixing and RTD

Patterns of material flow, mixing, viscous dissipation of mechanical energy, degree of filling and RT are much more complex and difficult to model in twin-screw extruders because of the extreme variability in screw configurations in the "tailor-made screw" technology. The effect of screw configuration and operating conditions on mixing and RTD has been studied extensively (Altomare and Ghossi 1986; Choudhury and Gautam 1998). Shear and thermal effects in a co-rotating twin-screw extruder were investigated by Chang and Halek (1991). Attempts to model and predict the effects of the screw configuration on these and other performance parameters have met with limited success, but directional indications are available from experimental studies.

Unlike the single-screw extruder, there is no continuous flow channel. The material passes from one screw to the other, and changes direction with each revolution (Fig. 12.13). Most of the material moves in C-shaped chambers delimited by the two screws and the barrel (Fig. 12.14). At any given time, different parts of the screw surface fulfill different functions (Sastrohartono et al. 1992). The part of the screw surfaces close to the surface of the barrel performs the action of translation, while mixing occurs in the intermeshing section between the screws. However, a good part of the mixing is done by special mixing and kneading

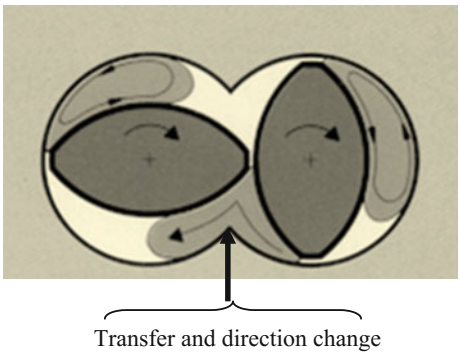

Fig. 12.13 Transfer of material from one screw to the other in twin-screw extruder

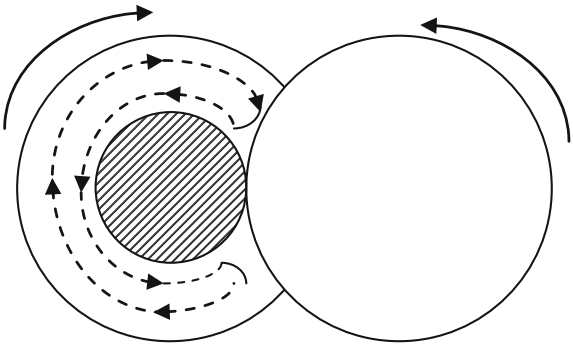

Fig. 12.14 Movement in C-shaped chambers (Adapted from Harper 1989)

elements on the screw, and depends on the number, length and spacing of the mixing regions. These elements are flat paddles without a significant restriction effect. Restriction is achieved mainly by gates and reverse-flow elements.

12.3 Food Extrusion Technologies

In this section, some of the commercially important and scientifically interesting food extrusion technologies are discussed.

12.3.1 Cold Extrusion—Pasta

Here we shall refer only to dry pasta, which is the principal industrial pasta product. Dry pasta is made by mixing wheat (preferably durum) semolina (Wiseman 2001) with water and other optional ingredients (eggs, tomato, spinach, vitamins, etc.), kneading the mixture to obtain a homogeneous dough, extruding this dough through die openings of the desired shape and drying the extrudate (Kill 2001; Brockway 2001). The most significant recent advances in pasta technology have been in the area of drying (which is outside the scope of the present chapter) and in the introduction of non-traditional ingredients into pasta products (e.g. Yalia and Manthey 2006; Marti et al. 2013).

The ingredients are pre-mixed in a screw or ribbon mixer. Sufficient water is added to bring the moisture content of the dough to about 30 %. The mixture is fed to the extruder, and a vacuum is applied to expel the air and prevent air bubbles in the product. The role of the extruder is twofold: kneading the dough and forming the product at the die (Dawe 2001). In contrast to cooking extrusion, cold extrusion is used for making pasta. Both single-screw and twin-screw extruders are used. The barrel is water-cooled so as to maintain the temperature of the dough around 40 °C. A lower temperature results in high dough viscosity, which makes passage through

the die difficult. A higher temperature results in degradation of the gluten and lower cooking quality (Abecassis et al. 1994). Pasta made by the older lamination technique is said to have a better texture, but most dry pasta today is made by extrusion.

12.3.2 Frozen Extrusion—Extruded Ice Cream

The application of extrusion to the production of ice cream in the late 1980s (Eisner 2006) is probably the most innovative recent development in food extrusion technology. Based on the research work of Windhab at ETH (Windhab and Wildmoser 2002), and further developed by Bollinger (Bollinger et al. 2000), this so-called low-temperature extrusion (LTE) ice cream technology is now being used by most leading manufacturers of ice cream. The mix is prepared, pasteurized and aged as usual, then partially frozen in a conventional ice cream freezer such as a swept-surface heat exchanger with air injection. A screw extruder with a strongly cooled barrel is used as a second freezer. Shear and heat transfer are controlled so as to promote the formation of very small ice crystals, in order to prevent the formation of large crystals and to distribute the fat evenly. The resulting ice cream is smooth and creamy. The material is extruded through dies of desired shapes and cuts. Thus, the LTE ice cream process not only produces ice cream of superior quality, but also helps form the final shape, such as bars or cone balls, without molds. The formed units are coated and sent to the final freezer for hardening.

12.3.3 Co-extrusion

Co-extrusion is the simultaneous extrusion of two or more materials of different properties (texture, flavor, color, etc.) in order to obtain a multi-phase product such as a filled breakfast cereal or snack (deCindio et al. 2002). Co-extrusion has been practiced in the polymer industry for many years, but its application in the food industry dates only from the 1980s. The two materials can come from two extruders. More commonly, one of the materials, usually a cereal-based mixture, is processed in a cooking extruder and constitutes the outer shell. The second material, usually a filling such as cheese, cream, jam or chocolate, is injected into the center of the cooked mass stream at the specially designed co-extrusion die (Figs. 12.15 and 12.16). A pump assembly (Fig. 12.17), is used to inject the filling at the appropriate pressure. The cereal-based shell material expands, while the colder filling does not. Down the production line, the malleable extrudate is crimped and cut. The choice of shell and filler materials is important. For example, in order to maintain the crispiness of the relatively hygroscopic shell, the moisture content of the filling must be low. Matching the viscosities of the dough and filling is necessary in order to minimize flow instabilities at the die.

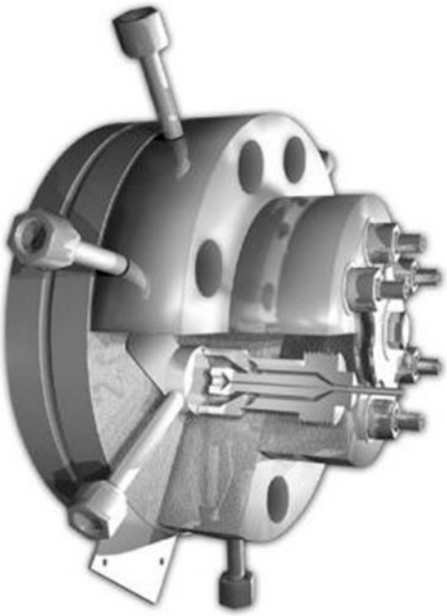

Fig. 12.15 Co-extrusion die (Courtesy of Baker Perkins Limited)

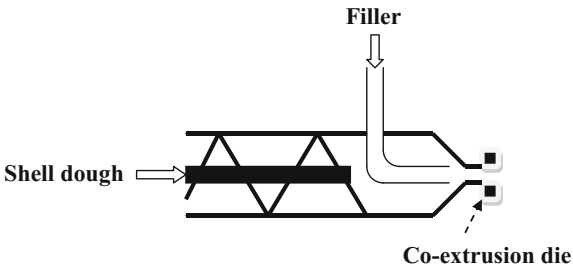

Fig. 12.16 Basic principle of co-extrusion

A special case of co-extrusion is co-injection, whereby a material of much lower viscosity (e.g. a food colorant in solution) is injected into the dough in order to incorporate ornamental patterns into the product (Morales-Alvarez and Rao 2012).

An interesting application of co-extrusion is the production of sausage by extrusion. Here, the outer shell material is a collagen gel, and the inner phase is extrusion-processed sausage meat. At the die, the collagen gel coats the cylinder of extruded meat as a thin film. A brine bath causes the collagen film to solidify and form an edible casing around the sausage "rope", which is cut to proper size and packaged. The casing can also be formed by a film of alginate, hardened by a calcium salt.

Fig. 12.17 Pump assembly for filler (Courtesy of Baker Perkins Limited)

12.3.4 Reactive Extrusion

Reactive extrusion refers to the use of extruders to carry out chemical or biochemical reactions. The advantages of the extruder as a continuous reactor with outstanding mixing capability led the chemical industry to consider it as a successful substitute for batch reactors, particularly for polymerization and polymer modification (Mani et al. 1999). The main food-related applications of reactive extrusion are hydrolysis and modification of starch (Linko 1992; Akdogan 1999; Xie et al. 2006). Reactive extrusion is particularly suited for the treatment of highly viscous media. Co-rotating twin-screw extruders are preferred for reactive extrusion, due to their efficient mixing, short and uniformly distributed RT and, above all, their ability to handle feeds with high water content (Akdogan 1999). Due to the high temperature, the efficient mixing and the relatively high concentration of the reactants, the reactions are very rapid and correspond well to the short RT in the extruder (Linko 1992).

Starch-related processes include gelatinization, liquefaction, saccharification and chemical modification (e.g. esterification). Gelatinization is essential for the solubilization of starch and for increasing the susceptibility of starch to enzymatic amylolysis. High temperature and sufficient water are necessary for gelatinization. In conventional processing, starch gelatinization requires water content of 35–40 %. Although gelatinization has been achieved by extrusion cooking at a moisture level as low as 10 % (Linko 1992), the process is accelerated by higher moisture, hence the advantage of the twin-screw extruder. Liquefaction refers to the initial stage of hydrolysis whereby the viscosity of starch paste is greatly reduced, producing non-sweet (low DE) syrups. The thermomechanical action of cooking extrusion, with or without enzymes, can be used for the continuous liquefaction of

starch (Davidson et al. 1984; Cheftel 1986; Colonna et al. 1989; Linko 1992; Karathanos and Saravacos 1992). The enzyme most commonly investigated in connection with starch liquefaction by reactive extrusion is thermostable α-amylase. Complete saccharification by extrusion alone is difficult, and the recommended procedure is extruder liquefaction in the presence of thermostable α-amylase, followed by post-extrusion hydrolysis by the enzyme glucoamylase.

Extrusion has been found to be a useful tool for carrying out starch-based reactions for the production of edible or biodegradable films (Carr 1991; Shogren 1996; Mani et al. 1999; Miladinov and Hanna 2000; Xie et al. 2006; Raquez et al. 2008; Li et al. 2011).

12.3.5 Texturization, Meat Analogs

Texturization of protein-rich vegetable sources to produce meat analogs was one of the first objectives of food extrusion (Clarck 1978). Texturized vegetable protein (TVP®, a trademark of the Archer Daniels Midland Company, USA) is made by extrusion cooking of defatted soybean flour. It is a sponge-like product with a lamellar structure. When rehydrated, it becomes chewable. In the late 1960s, TVP was widely used as a meat extender in a variety of products, and its consumption grew considerably after it was adopted by school lunch programs. It was also produced and distributed in a number of developing countries, with the objective of improving the protein nutrition of the population. In the beginning, single-screw extruders were used. TVP made of defatted soy flour has some of the characteristic beany flavor of the raw material. A better product was able to be made by texturizing soy protein concentrate (70 % protein) or isolated soybean protein (94–96 % protein). The flour or concentrate was extruded at a moisture content of about 20 % and temperature of 160–180 °C. After extrusion through a die of appropriate shape, the product was cut and dried (Berk 1992). The product was quite successful as a meat extender but not as a true meat analog, because it lacked the juiciness and fibrous structure of meat. Incidentally, a material similar to TVP was prepared by a non-extrusion process consisting of static compression, heating and pressure release, which led to the conclusion that "the working and kneading of the extrusion screw is not a prerequisite for the formation of texture" (Taranto et al. 1978). This view was contradicted by Holay and Harper (1982).

In the late 1980s, a process for making a better meat analog by extrusion was developed in Japan. Soybean protein isolate or concentrate is cooked in a twin-screw extruder at a moisture content of 60 % or higher and temperature of 100–150 ° C. (Noguchi 1989; Akdogan 1999). The material is extruded through a long, cooled die, which is essential for obtaining a fibrous structure. The addition of starchy components enhances fiber formation. According to Tolstoguzov et al. (1985), fiber formation is a consequence of protein aggregation following phase separation. Moisture content has a stronger influence on product characteristics than cooking temperature (Lin et al. 2002; Chen et al. 2010b). Water performs several

functions in the extrusion process. It acts as a lubricant, plasticizer and reaction reagent, lowers the glass transition temperature and causes different energy conversion ratios. As such, it affects the structure of the extrudate (Chen et al. 2010a). The molecular background of protein texturization has been investigated (Burgess and Stanley 1976; Aréas 1992; Ledward and Tester 1994). Based on the solubility of the extruded protein in various media, Prudencio-Ferrera and Arias (1993) concluded that disulfide bonds and non-covalent interactions are mainly responsible for the texture.

Soybean products are the principal but not the only material texturized by extrusion. Other protein-rich plant and animal sources have been treated by this process (Mégard et al. 1985; Hagan et al. 1986; Manoi and Rizvi 2009; Adhikari et al. 2009; Onwulata et al. 2010).

12.3.6 Direct Expanded Products

In terms of production volume, expanded snacks and breakfast cereals constitute the most widespread extruded food products, after pasta. Figure 12.18 shows a limited array of extruded snacks and breakfast cereals. Snacks and RTE breakfast cereals differ only in their formulation; their production processes are essentially the same.

Extruded foods, expanded by virtue of the pressure drop at the die exit, are known as second-generation or direct expanded products. Those obtained by thermal expansion during frying, oven or microwave heating of pellets (to be discussed in the next section) are called third-generation or indirect expanded products.

The standard production process of direct expanded foods is as follows:

The principal raw materials are cereal flours and starches. Optional additives are sugars, flavorings, colorants and fat. The raw materials and water are pre-mixed and fed into the extruder. Twin-screw extruders (Fig. 12.19) are used almost exclusively. Additional liquids may be added directly to the extruder. The mass is cooked at 160–180 °C and extruded through a die insert of appropriate shape. Expansion, both sectional and longitudinal, occurs at the exit from the die. The extrudate is cut to the desired length. At this point the product is still moist and soft. To make it crisp or crunchy, it must be dried. Heat-sensitive flavorings, vitamins and some fat may be added after extrusion. Some products are coated with sugar or cocoa and some are powdered or flaked.

The degree of expansion depends on the extrusion conditions as well as the properties of the raw materials. Literature on this matter up to 1989 was reviewed by Colonna et al. (1989). Starch is the principal factor in expansion. Nearly complete melting of the starch in the metering section is essential for expansion, and the viscosity of the melt at the die affects the degree of expansion. The amylose-to-amylopectin ratio is also important. The screw configuration has no marked effect on puffing (Sokheye et al. 1994). Payne et al. (1989) investigated the puffing of biological products in general, and classified the puffing processes into

Fig. 12.18 Some expanded snacks and breakfast cereals (Courtesy of Baker Perkins Limited)

Fig. 12.19 Pellet extruder (Courtesy of Baker Perkins Limited)

four categories based on the mechanism responsible for gas evolution: phase change, absorption, adsorption and chemical reaction. They defined an explosive expansion rate E, and concluded that E must be in the range of 0.05–12 m^3/s kg to puff biological products.

In addition to the production of snacks and breakfast cereals, extrusion expansion is being exploited for the production of fish feed (Oliveira et al. 1992). The possibility of producing sinking or floating particles by controlling the degree of expansion is attractive to manufacturers of feed for pisciculture.

12.3.7 Pellets—Products for Post-extrusion Expansion

One of the shortcomings of direct expanded snacks and breakfast cereals is their low bulk density, requiring expensive packaging and large storage space. Extrusion cooking is the first step in the production of pellets for subsequent puffing. Pellets are essentially non-porous particles of pre-cooked starchy materials. The raw materials are again starchy substances, optional additives, and water in sufficient quantity to permit starch gelatinization and adequate viscosity for regular transport in the extruder and through the die. Here, the only function of extrusion is cooking and forming. Expansion is avoided by pressure release and cooling before the die. The shape of the extrudate is determined by the die insert selected and the cutting device used. After extrusion, the pellets are stabilized by drying to a moisture content that warrants long-term conservation but leaves sufficient moisture (about

5–8 %) for puffing the pellet when exposed to high temperature. At a later stage, and often in a different location, the pellets are thermally puffed by frying, baking, roasting or microwave heating.

An important application of extrusion cooking without puffing is the production of raw materials for flaked breakfast cereals. In the production of the familiar corn flakes, for example, milled corn is moistened, then extrusion-cooked with other ingredients and extruded without expansion in the form of large particles. These granules are then flaked and toasted to produce corn flakes.

12.4 Nutrition, Safety, Availability

12.4.1 Effect of Extrusion on the Nutritional Characteristics of Foods

Chemical changes of nutritional significance in extrusion cooking have been extensively researched, and in-depth reviews of the voluminous literature on the subject are available (Cheftel 1986; Asp and Björck 1989; Singh et al. 2007). Extrusion cooking is essentially a thermal process. Consequently, any effect on the nutritional value depends primarily on the composition of the feed and the time–temperature history of the mass in the extruder. Design parameters and operating conditions such as the diameter and length of the extruder, screw configuration, mass flow rate, rotation speed and die geometry may have an effect on the nutritional properties in the measure that they affect the time–temperature profile. Thermal processes such as pasteurization, sterilization, cooking, roasting, frying, baking, blanching and drying are essential in the preparation of food industrially or at home. Therefore, the effect of extrusion cooking on nutrition should be evaluated in comparison to alternative thermal processes. Extrusion cooking is a high-temperature-short-time (HTST) process, and as a rule, HTST processes produce organoleptically and nutritionally superior food.

(a) Proteins: In general, extrusion improves the digestibility of proteins. This is attributed in great part to the inactivation of inhibitors of proteolysis in the GI tract (Asp and Björck 1989; Alonso et al. 2000). Acceleration of the Maillard reaction resulting in the depression of lysine availability is of concern, but can be minimized by extruding at moderate temperatures, below 180 °C (Cheftel 1986), and excluding reducing sugars.

(b) Digestible carbohydrates: These include mono-, di- and some oligosaccharides, dextrins and starch. Nutrition-wise, the most important change is the gelatinization of starch, which is efficiently performed by extrusion cooking, even at low moisture content. Gelatinization facilitates starch digestion by amylases in the GI tract.

(c) Non-digestible carbohydrates: These include cellulose, hemicelluloses, pectin and some polysaccharides. Many of these are components of the large and

nutritionally important group known as dietary fiber. Most raw materials subjected to extrusion cooking, such as cereal flours, are important sources of dietary fiber (Brennan et al. 2008) Extrusion cooking at moderate conditions does not significantly alter the dietary fiber content, but solubilizes some of it (Singh et al. 2007). Extrusion destroys some of the flatulence-inducing non-digestible oligosaccharides such as raffinose and stachyose (Asp and Björck 1989).

(d) Lipids: High fat content is generally avoided in the formulation of extruded foods, mainly because of the excessive lubrication and the resulting cylindering effect of fats. Extrusion cooking may induce slight and nutritionally insignificant chemical changes in characteristics such as unsaturated/saturated ratio and *trans* fatty acid content (Asp and Björck 1989).

(e) Vitamins: Heat-labile vitamins such as thiamine and ascorbic acid are degraded to a considerable extent by extrusion cooking. The rate of degradation is comparable to that caused by similar thermal processes. The most common practice for replacing the lost vitamins in products where vitamin content is important (e.g. RTE breakfast cereals) is post-extrusion incorporation.

12.4.2 Safety and Stability of Extruded Foods

As an HTST thermal process, extrusion should be considered as one of the most efficient food stabilization processes. The time–temperature profile of extrusion cooking, even under "moderate" conditions, is sufficient for the inactivation of most spoilage enzymes. The same is true for microorganisms and their spores (van de Velde et al. 1984). In a study investigating the destruction of one of the most heat-resistant spoilage microorganisms, *Bacillus stearothermophilus* (used for the validation of sterilization cycles in autoclaves, and now renamed *Geobacillus stearothermophilus*) (Bouveresse et al. 1982), no living spores were left after extrusion at 165 °C, provided that the average RT was at least 80 s. Cheftel (1989) calculated that the time–temperature profile of typical extrusion processes exceeded the thermal treatment needed to achieve commercial sterilization in foods. It is interesting to note that one of the reasons for suggesting extrusion cooking as a replacement for the lengthy process of conching (Aguilar et al. 1995) was the ability of extrusion to pasteurize the cocoa mass.

Extrusion cooking destroys many antinutritional and toxic factors that are frequently present in the raw materials treated. Inactivation of trypsin inhibitors in full-fat soybean flour was demonstrated by Mustakas et al. (1964). Destruction of antinutritional factors in animal feeds was one of the objectives in developing low-cost extruders in the 1960s. Trypsin inhibitors and lectins in pulses are also efficiently inactivated by extrusion (Cheftel 1989). Reduction of flatus-inducing sugars was mentioned in a previous section.

Mycotoxins are relatively heat-resistant toxic substances produced by molds. Kabak (2009) discusses the destruction of mycotoxins by extrusion. Cooking extrusion causes considerable degradation of aflatoxin in corn and peanuts (Saalia and Phillips 2011a, b), without adversely affecting the nutritional quality of the meal.

On the negative side, as far as food safety is concerned, the risk of the formation of toxic factors as a result of heating amino acids to high temperatures should be considered. The risk of acrylamide formation is of particular concern (Sharp 2003; Singh et al. 2007). Acrylamide, a by-product of the Maillard reaction, is a "probable human carcinogen", produced when certain foods are heated to high temperatures. The main precursor is the amino acid asparagine, in the presence of carbohydrates. Acrylamide is produced by industrial and home cooking alike, when asparagine-rich foods (such as potatoes) are subjected to high temperatures (such as frying). Acrylamide is present in potato and cereal foods produced by extrusion cooking (Singh et al. 2007; Mulla et al. 2011) and in extruded pellets fried for post-extrusion expansion. It is not yet known whether the concentrations of acrylamide typically found in extruded foods constitute a health risk for humans. At present, there are no guidelines or regulations concerning acrylamide in food. Techniques for reducing acrylamide levels in foods are available or in development, most of which are based on the enzymatic decomposition of asparagine.

12.4.3 Food Availability

The process of extrusion is probably the best example of the significant contribution of food engineering to the global availability of food. Pasta products are the backbone of nutrition in Italy and a significant factor in food availability elsewhere. RTE breakfast cereals are indispensable components of the diet of modern society, where convenience is the chief parameter for the selection of foods. For populations and individuals who cannot afford meat or would like to reduce their meat consumption for economic, religious or environmental reasons, texturized vegetable proteins offer a valuable solution.

Extrusion cooking has been suggested as a means of transforming underutilized plants and animals, by-products and waste material into potential food resources and as a way of reducing production costs. The use of extrusion cooking for the production of tarhana, a traditional Turkish staple based on wheat and yogurt, offers economic advantages (Ibanoğlu and Ainsworth 2010). Nixtamal is the lime-treated corn used for making tortillas. The tedious process of nixtamal production from corn can be replaced by extrusion cooking to save time and energy (Mensah-Agyapong and Horner 1992). Certain kinds of pulses are not fully utilized because they are difficult to cook. Extrusion cooking has been suggested as a solution to the problem (Ruiz-Ruiz et al. 2008). Sources of animal protein that can be upgraded by extrusion include fish (Bhattacharya et al. 1992; Choudhury and Gogoi 1995) and poultry (Mégard et al. 1985).

References

Abecassis J, Abbou R, Chaurand M, Morel MH, Vernoux R (1994) Influence of extrusion condition on extrusion speed, temperature and pressure in the extruder and on pasta quality. Cereal Chem 71:247–253

Adhikari K, Cole A, Grün I, Heymann H, Hsieh F-H, Huff H (2009) Physical and sensory characteristics of processed cheeses manufactured by extrusion technology. J Sci Food Agric 89:1428–1433

Aguilar CA, Dimick PS, Hollender R, Ziegler GR (1995) Flavor modification of milk chocolate by conching in a twin-screw, co-rotating, continuous mixer. J Sens Stud 10:369–380

Akdogan H (1999) High moisture food extrusion. Int J Food Sci Technol 34:195–207

Alonso R, Aguirre A, Marzo F (2000) Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. Food Chem 68:159–165

Altomare RE, Ghossi P (1986) An analysis of residence time distribution patterns in a twin screw cooking extruder. Biotechnol Prog 2:157–163

Arêas JA (1992) Extrusion of food proteins. Crit Rev Food Sci Nutr 32:365–392

Asp N-G, Björck I (1989) Nutritional properties of extruded foods. In: Mercier C, Linko P, Harper JM (eds) Extrusion cooking. Amer. American Association of Cereal Chemists, Inc., St. Paul

Ayoub A, Rizvi SSH (2011) Reactive supercritical fluid extrusion for development of moisture resistant starch-based foams. J Appl Polym Sci 120:2242–2250

Berk Z (1992) Technology of production of edible flours and protein products from soybeans. FAO, Rome

Berk Z (2013) Food process engineering and technology, 2nd edn. Academic Press, London

Bhattacharya S, Das H, Bose AN (1992) Rheological behaviour during extrusion of blends of minced fish and wheat flour. J Food Eng 15:123–137

Bigg DM, Middleman S (1974) Mixing in a screw extruder. A model for residence time distribution and strain. Ind Eng Chem Fundam 13:66–71

Bimbenet JJ, Duquenoy A, Trystram G (2002) Génie des Procédés Alimentaires. Dunod, Paris

Bolliger SB, Kornbrust HD, Goff B, Tharp W, Windhab EJ (2000) Influence of emulsifiers on ice cream produced by conventional freezing and low-temperature extrusion processing. Int Dairy J 10:497–504

Bounié D (1988) Modelling of the flow pattern in a twin-screw extruder through residence time distribution experiments. J Food Eng 7:223–246

Bouveresse JA, Cerf O, Guilbert S, Cheftel JC (1982) Influence of extrusion cooking on the thermal destruction of *Bacillus stearothermophilus* spores in a starch-protein-sucrose mix. LWT 15:135–138

Brennan MA, Monro JA, Brennan CS (2008) Effect of inclusion of soluble and insoluble fibres into extruded breakfast cereal products made with reverse screw configuration. Int J Food Sci Technol 43:2278–2288

Brockway BE (2001) Pasta. In: Dendy DAV, Dobraszczyk BV (eds) Cereal and cereal products chemistry and technology. Aspen Publishers, Gathersbury

Bruin S, Van Zuilichem DJ, Stolp W (1978) A review of the fundamental and engineering aspects of extrusion of biopolymers in a single-screw extruder. J Food Proc Eng 2:1–37

Burgess GD, Stanley DW (1976) A possible mechanism for thermal texturization of soybean protein. Can Inst Food Sci Technol 9:228–230

Camire ME (1998) Chemical changes during extrusion cooking. Recent advances. Adv Exp Med Biol 434:109–121

Carley JF, Mallouk RS, McKelvey JM (1953) Simplified flow theory for screw extruders. Ind Eng Chem 45:974–978

Carr M (1991) Starch-derived glycol and glycerol glucosides prepared by reactive extrusion processing. J Appl Polym Sci 42:45–53

Chang KLB, Halek GW (1991) Analysis of shear and thermal history during co-rotating twin-screw extrusion. J Food Sci 56:518–531
Cheftel JC (1986) Nutritional effects of extrusion cooking. Food Chem 20:263–283
Cheftel JC (1989) Extrusion cooking and food safety. In: Mercier C, Linko P, Harper JM (eds) Extrusion cooking. American Association of Cereal Chemists, Inc., St. Paul
Chen FL, Wei YM, Zhang B (2010a) System parameters and product properties response of soybean protein extruded at wide moisture range. J Food Eng 96:208–213
Chen FL, Wei YM, Zhang B (2010b) Characterization of water state and distribution in textured soybean protein using DSC and NMR. J Food Eng 100:522–526
Choudhury GS, Gogoi BK (1995) Extrusion processing of fish muscle: a review. J Aquat Food Prod Technol 4:37–67
Choudhury GS, Gautam A (1998) On-line measurement of residence distribution in a food extruder. J Food Sci 63:529–534
Cisneros FH, Kokini JL (2002) Effect of extrusion operating parameters on air bubble entrapment. J Food Proc Eng 25:251–283
Clarck JP (1978) Texturization by extrusion. J Texture Stud 9:109–123
Colonna P, Tayeb J, Mercier C (1989) Extrusion cooking of starch and starchy products. In: Mercier C, Linko P, Harper JM (eds) Extrusion cooking. American Association of Cereal Chemists, Inc., St. Paul
Crowley PR (1979) Transfering LEC technology to developing countries: from concept to application and beyond. In: Wilson DE (ed) Low-cost extrusion cookers. USDA, Fort Collins
Davidson VJ, Paton D, Diosady LL, Laroque G (1984) Degradation of wheat starch in a single-screw extruder: characteristics of extruded starch polymers. J Food Sci 49:453–458
Dawe PR (2001) Pasta mixing and extrusion. In: Kill RC, Turnbull K (eds) Pasta and semolina technology. Blackwell Scientific Ltd., Oxford
deCindio B, Gabriele D, Pollini CM, Peressini D, Sansidoni A (2002) Filled snacks production by coextrusion cooking: 1- rheological modeling of the process. J Food Eng 62:67–74
deMesa-Stonestreet NJ, Alavi S, Gwirtz J (2012) Extrusion-enzyme liquefaction as a method for producing sorghum protein concentrates. J Food Eng 108:365–375
Eisner MD (2006) Fat structure development in low temperature extruded ice cream. Doctoral thesis No. 16757. ETH, Zurich
Ferdinand JM, Lai-Fook RA, Ollet AL, Smith AC, Clark SA (1990) Structure formation by carbon dioxide injection in extrusion cooking. J Food Eng 11:209–224
Frame ND (1994) The technology of extrusion cooking. Chapman & Hall, New York
Guy R (2001) Extrusion cooking: technologies and applications. Woodhead Publishing, Cambridge
Hagan RC, Dahl SR, Villota R (1986) Texturization of co-precipitated soybean and peanut proteins by twin-screw extrusion. J Food Sci 51:367–370
Harper JM (1978) Food extrusion. Crit Rev Food Sci Nutr 11:155–215
Harper JM (1980) Food extrusion. CRC Press, Boca Raton
Harper JM (1989) Food extruders and their applications. In: Mercier C, Linko P, Harper JM (eds) Extrusion cooking. American Association of Cereal Chemists, Inc., St. Paul
Harper JM (1992) A comparative analysis of single and twin-screw extruders. In: Kokini JL, Ho C-T, Karwe MV (eds) Food extrusion science and technology. Marcel Dekker, New York
Holay SH, Harper JM (1982) Influence of the extrusion shear environment on plant protein texturization. J Food Sci 47:1869–1874
Ibanoğlu Ş, Ainsworth P (2010) Energy considerations on extrusion of tarhana, a wheat flour based product. J Food Proc Eng 33:448–465
Kabak B (2009) The fate of mycotoxins during thermal food processing. J Sci Food Agric 89:549–554
Karathanos VT, Saravacos GD (1992) Water diffusivity in the extrusion cooking of starch materials. In: Kokini JL, Ho C-T, Karwe MV (eds) Food extrusion science and technology. Marcel Dekker, New York

Kazemzadeh M (2012) Introduction to extrusion technology. In: Maskan M, Altan A (eds) Advances in food extrusion technology. CRC Press, Boca Raton
Kill RC (2001) Introduction. In: Kill RC, Turnbull K (eds) Pasta and semolina technology. Blackwell Scientific Ltd. Oxford
Kokini JL (1993) The effect of processing history on chemical changes in single- and twin-screw extruders. Trends Food Sci Technol 4:324–329
Kumar P, Sandeep KP, Alavi S, Truong VD, Gorga RE (2010) Preparation and characterization of bio-nanocomposite films based on soy protein isolate and montmorillonite using melt extrusion. J Food Eng 100:480–489
Ledward DA, Tester RF (1994) Molecular transformations of proteinaceous foods during extrusion processing. Trends Food Sci Technol 5:117–120
Li M, Liu P, Zou W, Yu L, Xie F, Pu H, Liu H, Chen L (2011) Extrusion processing and characterization of edible starch films with different amylose contents. J Food Eng 106:95–101
Linko P (1992) Twin-screw extrusion cooker as a bioreactor for starch processing. In: Kokini JL, Ho C-T, Karwe MV (eds) Food extrusion science and technology. Marcel Dekker, New York
Lin S, Huff HE, Hsieh F (2002) Extrusion process parameters, sensory characteristics and structural properties of a high moisture soy protein meat analog. J Food Sci 67:1066–1072
Mani R, Battacharya M, Tang J (1999) Functionalization of polyesters with maleic anhydride by reactive extrusion. J. Polym Sci A Polym Chem 37:1693–1702
Manoi K, Rizvi SSH (2009) Physicochemical changes in whey protein concentrate texturized by reactive supercritical fluid extrusion. J Food Eng 95:627–636
Marti A, Caramanico R, Bottega G, Pagani A (2013) Cooking behavior of rice pasta: effect of thermal treatments and extrusion conditions. LWT 54:229–235
Mégard D, Kitabatake N, Cheftel JC (1985) Continuous restructuring of mechanically deboned chicken meat by HTST extrusion-cooking. J Food Sci 50:1364–1369
Mensah-Agyapong J, Horner WFA (1992) Nixtamalisation of maize (*Zea mays* L) using a single screw cook-extrusion process on lime-treated grits. J Sci Food Agric 60:509–514
Mercier Ch, Cantarelli C (1986) Introductory remarks. In: Mercier C, Cantarelli C (eds) Pasta and extrusion cooked foods. Elsevier, New York
Meuser F, Wiedmann W (1989) Extrusion plant design. In: Mercier C, Linko P, Harper JM (eds) Extrusion cooking. American Association of Cereal Chemists, Inc., St. Paul
Miladinov VD, Hanna MA (2000) Starch esterification by reactive extrusion. Ind Crops Prod 11:51–57
Morales-Alvarez J, Rao M (2012) Industrial application of extrusion for development of snack products including co-injection and pellet technologies. In: Medeni M, Altan A (eds) Advances in food extrusion technology. CRC Press, Boca Raton
Mustakas GC, Griffin EL, Allen IE, Smith OB (1964) Production and nutritional evaluation of extrusion-cooked full-fat soybean flour. J Amer Oil Chem Soc 41:607–614
Mulla MZ, Bharadwaj VR, Uday S, Annapure US, Singhal RS (2011) Effect of formulation and processing parameters on acrylamide formation: a case study on extrusion of blends of potato flour and semolina. LWT 44:1643–1648
Mulvaney SJ, Rizvi SHS (1993) Extrusion processing with supercritical fluids. Food Technol 47:74–82
Noguchi A (1989) Extrusion cooking of high-moisture protein foods. In: Mercier C, Linko P, Harper JM (eds) Extrusion cooking. American Association of Cereal Chemists, Inc., St. Paul
Oliveira MA, Møller-Holst S, Haaland H, Roselund G (1992) The effect of process parameters on expansion of extruded fish feeds. In: Kokini JL, Ho C-T, Karwe MV (eds) Food extrusion science and technology. Marcel Dekker, New York
Onwulata CI, Phillips JG, Tunick MH, Qi PX, Cooke PH (2010) Texturized dairy proteins. J Food Sci 75:E100–E109
Payne FA, Taraba Jl, Saputra D (1989) A review of puffing processes for expansion of biological products. J Food Eng 10:183–197
Pinto G, Tadmor Z (1970) Mixing and residence time distribution in melt scre extruders. Polym Eng Sci 10:279–288

Prudencio-Ferreira SH, Areas JG (1993) Protein-protein interactions in the extrusion of soya at various temperatures and moisture contents. J Food Sci 58:378–381
Raquez J-M, Nabar Y, Srinivasan M, Shin BY, Narayan R, Dubois P (2008) Maleated thermoplastic starch by reactive extrusion. Carbohydr Polym 74:159–169
Riaz MN (2000) Extruders in food applications. CRC Press, Boca Raton
Ruiz-Ruiz J, Martınez-Ayala A, Drago S, Gonzalez R, Betancur-Ancona D, Chel-Guerrero L (2008) Extrusion of a hard-to-cook bean (*Phaseolus vulgaris* L.) and quality protein maize (*Zea mays* L.) flour blend. LWT 41:1799–1807
Saalia FK, Phillips RD (2011a) Degradation of aflatoxins by extrusion cooking: effects on nutritional quality of extrudates. LWT 44:1496–1501
Saalia FK, Phillips RD (2011b) Reduction of aflatoxins in peanut meal by extrusion cooking in the presence of nucleophiles. LWT 44:1511–1516
Sastrohartono T, Karwe MV, Jaluria Y, Kwon TH (1992) Numerical simulation of fluid flow and heat transfer in a twin-screw extruder. In: Kokini JL, Ho C-T, Karwe MV (eds) Food extrusion science and technology. Marcel Dekker, New York
Sauceau M, Fages J, Common A, Nikitine C, Rodier E (2011) New challenges in polymer foaming: a review of extrusion processes assisted by supercritical carbon dioxide. Prog Polym Sci 36:749–766
Sharp D (2003) Acrylamide in food. Lancet 361(9355):361–363
Shogren RL (1996) Preparation, thermal properties, extrusion of high amylose starch acetates. Carbohydr Polym 29:57–62
Singh S, Gamlath S, Wakeling L (2007) Nutritional aspects of food extrusion: a review. Int J Food Sci Technol 42:916–929
Sokhey AS, Kollengode AN, Hanna MA (1994) Screw configuration effects on corn starch expansion during extrusion. J Food Sci 59:895–896
Steinmacher NC, Honna FA, Gasparetto AV, Anibal D, Grossmann MVE (2012) Bioconversion of brewer's spent grains by reactive extrusion and their application in bread-making. LWT 46:542–547
Tadmor Z, Gogos C (1979) Principles of polymer processing. Wiley, New York
Taranto MV, Cegla GF, Rhee KC (1978) Morphological, ultrastructural and rheological evaluation of soy and cottonseed flours texturized by extrusion and nonextrusion processing. J Food Sci 43:973–979
Tayyeb J, Della Valle G, Barres C, Vergnes B (1992) Simulation of transport phenomena in twin-screw extruders. In: Kokini JL, Ho C-T, Karwe MV (eds) Food extrusion science and technology. Marcel Dekker, New York
Tolstoguzov VB, Grinberg VY, Gurov AN (1985) Some physic-chemical approaches to the problem of protein texturization. J Agric Food Chem 33:151–159
Ullrich M (2008) Historical development of co-rotating twin-screw extruders. In: Kohlgrüber K (ed) Co-rotating twin screw extruders. Carl Hansen Verlag, Münich
Van de Velde C, Bounie D, Cuq JL, Cheftel JC (1984) Destruction of microorganisms and toxins by extrusion-cooking. In: Zeuthen P, Cheftel JC, Eriksson C, Jul M, Leniger H, Linko P, Varela G, Vos G (eds) Thermal processing and quality of foods. Elsevier, London
Van Zuilichem DJ, de Swart JG, Buisman G (1973) Residence time distribution in an extruder. LWT 6:184–188
Wang YY, Ryu GH (2013) Physical properties of extruded corn grits and corn fibre by CO_2 injection extrusion. J Food Eng 116:14–20
Windhab EJ, Wildmoser H (2002) Extrusion: a novel technology for the manufacture of ice cream. Bull IDF 374:43–49
Wiseman G (2001) Durum wheat. In: Kill RC, Turnbull K (eds) Pasta and semolina technology. Blackwell Scientific Ltd., Oxford
Xie F, Yu L, Liu H, Chen, L (2006) Starch modification using reactive extrusion. Starch Stärke 58:131–139
Yacu WA (1985) Modeling a twin screw co-rotating extruder. J Food Proc Eng 8:1–21

Yalia SR, Manthey FA (2006) Effect of semolina and absorption level on extrusion of spaghetti containing non-traditional ingredients. J Food Sci Agric 86:841–848
Yeh I-E, Jaw Y-M (1998) Modeling residence time distributions for single screw extrusion process. J Food Eng 35:211–232
Zhao X, Wei Y, Wang Z, Chen F, Ojokoh AO (2011) Reaction kinetics in food extrusion: methods and results. Crit Rev Food Sci Nutr 51(9):835–854

Chapter 13
Nonthermal Stabilization Processes

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Abstract The processing of food by nonthermal technologies—such as high pressure, pulsed electric fields, and ultrasound—is gaining relevance within the food industry. Fresh-tasting foods along with minimal impact on nutritional attributes, low energy consumption, minimal and fast processing, environmental-friendliness, effectiveness at inactivating pathogenic and spoilage microorganisms some of the characteristics making these technologies so attractive. This chapter focuses on the affect of selected nonthermal-processing technologies on the stability of bioactive compounds in a number of foods. A brief description of these technologies is also included.

Keywords Nonthermal processing · Stability of bioactive compounds · High pressure · Pulsed electric fields · Ultrasound

13.1 Introduction

Current consumer demands for fresh and minimally processed food products that provide health promotion or disease prevention indicate that new food-processing systems should be designed toward higher-quality food products.

The most common technologies used for conventional food processing in order to inactivate microorganisms and enzymes in food products—such as pasteurization, blanching, and sterilization—may degrade beneficial heat-sensitive com-

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pounds such as aroma molecules, vitamins and nutrients. Unfortunately, treatment time and processing temperature are also proportional to the amount of bioactive compounds lost, and in some cases the production of significant undesirable flavors and colors can take place (Bermúdez-Aguirre et al. 2011; Feng et al. 2011; Bermúdez-Aguirre and Barbosa-Cánovas 2012). Today, scientists and the food industry are focusing on process development for applying nonthermal technologies that are not only capable of inactivating undesired microorganisms but also preserving bioactive compounds and physicochemical characteristics.

Nonthermal technologies are becoming prevalent in the food industry as an alternative to conventional processes. Nonthermal-processing technologies can in fact be used to inactivate microorganisms and enzymes, to extract bioactive compounds, and to deliver structural changes in the food matrices. Because temperature is not the governing factor in processing, these technologies have less influence on the loss of heat-sensitive bioactive compounds.

Environmental friendliness, low energy requirements and better retention of nutrients are some of the characteristics making these technologies popular. Such technologies are also referred to as "minimal-processing technologies" due to their ability to preserve the sensory attributes of food in order to them characteristics similar to those of fresh food products.

In this chapter, the effect of nonthermal technologies—such as ultrasound, pulsed electric fields, high hydrostatic pressure, cold plasma, and some combinations of these—will be described in terms of their effect on the stability of bioactive compounds through a comparative analysis of the main studies that have been reported in recent literature. In particular, an overview of the application of nonthermal technologies in the food industry will be provided by presenting the background of each of them and its principles as well as addressing their contribution in the preservation of bioactive compounds in food systems.

13.2 Ultrasound

Sound waves travel in solid, liquid, or gaseous media, and the characteristics of the medium influence how the waves spread and how they are perceived. Ultrasound waves are higher than the threshold of human hearing (>14–20 kHz) and can be classified according to their frequency: high (5–10 MHz) or low (20–100 kHz) (Soria and Villamiel 2010; Chandrapala et al. 2012). High-frequency waves are used mainly for medical diagnosis, whereas low-frequency waves are used in food processing. Ultrasound for food applications is classified into low (0.1–1 W/cm^2) and high power (10–1000 W/cm^2). The intensity of ultrasound indicates the quantity of energy applied per unit area. Low-intensity ultrasound (using high frequency and low power) has been widely used as a non-invasive analytical technique to provide information on the physicochemical properties of food such as firmness, ripeness, sugar content, and acidity (Soria and Villamiel 2010; Demirdöven and Baysal 2008; Chandrapala et al. 2012; Anese et al. 2013). Low-frequency and high-power ultrasound is used in the

modification and alteration of either the physical or chemical properties of food; in addition, high intensity has been used in microbiological inactivation (McClements 1995; Soria and Villamiel 2010; Anese et al. 2013).

Ultrasound waves pass through a liquid/fluid medium, thus producing mechanical vibration (Ashokkumar 2011). Displacement of sound waves into a liquid in combination with dissolved gas in the medium leads to formation of thousands of bubbles. The phenomenon in which the bubbles grow and collapse by ultrasonic effect is known as "acoustic cavitation" (Leighton 1998; Chemat and Khan 2011; Louisnard and González-García 2011).

In compression processes, the bubbles collapse or implode when they grow above a critical size, in order to return to their original size, thus generating the cavitation phenomenon. During cavitation, and pressure changes quickly and temperature increases slightly (Soria and Villamiel 2010; Ashokkumar 2011). These changes imply energy liberation, thus leading to the formation of micro-turbulences and micro-currents in micro-environmental systems. The characterization of bubbles under acoustic cavitation has been described in detail by Ashokkumar (2011) in terms of the growth process, temperature changes, size, and life time of bubbles.

One of the most important parameters to be controlled during ultrasound treatment is temperature because there is a rapid increase in temperature during treatment, which may cause the degradation of bioactive compounds. It has also been reported that the cavitation phenomenon is reduced with an increase in temperature (Sango et al. 2014). Thus, the effectiveness of ultrasound as an eradicator of microorganisms could be reduced.

Ultrasound seems to be a promising alternative processing technology for food while preserving its physicochemical characteristics. However, some studies using ultrasound reflect the production of flavors in the food (Marchesini et al. 2012). Ultrasound works in food stability either by inactivating microorganisms and enzymes or by enhancing the extraction of bioactive compounds. If the food has had, incidentally or not, contact with microorganisms, the application of ultrasound waves could be an effective option to eradicate them. In bacterial cells ultrasound causes permeabilization of the cell membranes, surface erosion, and the rubbing of cells, thus resulting in cell death with minor changes in quality. Many research papers about the inhibition of cells have discussed this change (Cameron et al. 2008; Bermúdez-Aguirre et al. 2011; Bermúdez-Aguirre and Barbosa-Cánovas 2012; Moody et al. 2014).

The ultrasound process works at low temperature and over a short time, which allows high retention of bioactive compounds in food materials during treatment. Table 13.1 lists some of the results reported during ultrasound processing, in which minimal changes in bioactive compounds are reported.

Different methodologies and control parameters in ultrasound processing—such as types of ultrasound (in bath, submersion in a reactor), modus (intermittent and continuous pulsation), frequency and power levels, time, temperature, and ultrasound combined with other technologies such as UV application—have been reported. These differences show a high influence on bioactive compound percentage retention, and in most of the studies a good retention percentage has been reported.

Table 13.1 Application of ultrasound for the stabilization of bioactive compounds

Matrix	About compounds and other implications	Process	Processing conditions			References
			Intensity and frequency	Temperature (°C)	Time (min)	
Air-dried pears	Saccharides and bioactive compounds	Ultrasound in bath	Power: 200 W Frequency: 37 kHz	21	0, 10, 22, 35 and 45	Komes et al. (2013)
Peanuts	Enhancement of phenolics and antioxidants	Combinations of ultrasound (US) UV treatments	Frequency: 20 kHz Power: 750 W Amplitude: 21, 48, and 68 %	25	NR	Sales and Resurreccion (2010a)
Peanuts	Phenolics, antioxidants, and sensory acceptance	Combinations of ultrasound (US)–UV treatments	Power density: 25, 50 and 75 mW/cm^3	25	2, 5, and 8	Sales and Resurreccion (2010b)
Peanuts	Resveratrol, piceid, and total stilbenes	Combinations of ultrasound (US)-UV treatments	Power density: 25, 50 and 75 mW/cm^3	25	2, 5, and 8	Sales and Resurreccion (2009)
Fruit smoothies	Bioactive, quality and rheological characteristics	Ultrasound pulsing	Frequency: 20 kHz Power density: 9.24,13.28, and 22.79 W/cm^2 Amplitude: 24.4, 42.7 and 61 micro	25 ± 1.0	3–10	Keenan et al. (2012)

NR Not reported

In many studies, vitamin C is mentioned as one of the important quality parameters in fruit and vegetable juices because this vitamin has antioxidant activity. Many reports show a 60 % retention of bioactive compounds after ultrasound processing, whereas other reports show a slightly lower percentage. This might be due to the reaction of bioactive compounds with oxygen. Some research shows that sonication is responsible for an increase in antioxidant activity and in the content of bioactive compounds. Bhat et al. (2011) reported an increase in ascorbic acid, total phenolic content, and antioxidant activity after ultrasound treatment. Elimination of dissolved oxygen during cavitation resulted in an increase in ascorbic acid, which could otherwise cause its degradation. An increase in phenolic compounds is caused by the addition of sonochemically generated hydroxyl radicals to the aromatic ring of the phenolic compounds. An increase in antioxidant activity could be due to the extraction process, which may cause vegetable-cell disruption, thus releasing bioactive compounds such as pigments and other antioxidant molecules.

Improving the physicochemical characteristics of foods by the creation of emulsions is another application of ultrasound (Abbas et al. 2013). Emulsion processing could be carried out with high-pressure homogenizers, micro-fluidizers, mixers, etc. Ultrasonic emulsification offers several benefits compared with conventional methods. The energy required to produce an emulsion by ultrasound is less than that needed in conventional methods; emulsions generated by ultrasound are more stable, require minimum time, and have a narrow particle-size distribution (Chemat and Khan 2011).

Table 13.2 Emulsions generated by ultrasound of bioactive compounds

Active ingredient	Process conditions				References
	Frequency (kHz)	Power (W)	Temperature (°C)	Time (min)	
Curcumin	20	10, 20, 30, 40, 50, 60, and 70	40–45	1–13	Abbas et al. (2014)
Flaxseed oil	20	88, 132, and 176	22.5	1–8	Shanmugam and Ashokkumar (2014)
Canthaxanthin	25	200	<20	4	Gharibzahedi et al. (2013)
Basil (*Ocimumbasilicum)* oil	20	750	NR	NR	Ghosh et al. (2013a)
Cinnamon (*Cinnamomum zeylanicum*) oil	20	750	NR	NR	Ghosh et al. (2013b)
Eucalyptus oil	NR	NR	NR	10	Saranya et al. (2012)
Cheese	20	120–200	50	20	Mongenot et al. (2000)

NR Not reported

Emulsification by ultrasound is also attributed to the cavitation phenomenon. The waves in ultrasound generate cavitation bubbles, which collapse at or near the oil water interface, thus causing disruption and mixing, which results in the formation of very fine emulsions (Anton et al. 2008; Chandrapala et al. 2012). Ultrasonic emulsification delays phase separation in multi-component systems and therefore improves stability to incorporate bioactive compounds in emulsions. Several studies have been carried out using ultrasonic emulsification processes to produce nano-emulsions containing bioactive compounds with enhanced stability and shelf life as listed in Table 13.2.

13.3 Pulsed Electric Fields

Processing by pulsed electric fields (PEF) involves the passage of pulses with high voltage to the food placed between two electrodes (Zhang et al. 1995; Sobrino-López and Martín-Belloso 2010). It has been successfully applied to liquids foods such as juice, milk, etc. as well as semisolid and solid foods including meat and seafood. PEF systems consist of a high-voltage pulse generator, capacitor banks, a switch, and treatment chambers. Electrodes conduct the high-intensity electrical pulse to the food where it receives a force-per-unit charge, which is responsible for microbial inactivation through dielectric breakdown of the cell membrane; this process also leads to the extraction of intracellular components from the food.

Induced electric fields can be applied in a medium flowing in a continuous system or in a stepwise circulation mode, in which the liquid returns to the container without interruption (Sobrino-López and Martín-Belloso 2010). The duration of single or repetitive pulses is in the range between nanoseconds and milliseconds. The main parameters involved in PEF technology are electric-field intensity, temperature, pulse wave (form, shape, frequency, and width), treatment time, chamber design, and physical properties of the food such as electrical conductivity (Jin et al. 2015). Every parameter should be monitored during treatment in order to have better control during the process. Nevertheless, temperature and the presence of bubbles in flow media are determinant factors influencing the effective discharge of pulses. Temperature can modify the electrical conductivity, density, viscosity, and thermal conductivity of the material; as a consequence, variable electric-field distribution in the matrix can take place also. In addition, the bubbles can distort the electric field and may cause arcing in the system (Sobrino-López and Martín-Belloso 2010).

Among other nonthermal technologies, PEF is a promising technology due to its capacity to inhibit microorganisms with high effectiveness giving the fresh-like characteristics. The use of high-intensity pulsed electric fields (between 10 and 80 kV/cm) causes the disruption of membrane cells due to electroporation; this refers to the creation of pores when electric pulses are applied to cells and, as a consequence, lysis of cells takes place, although cells might return to their original state (Castro et al. 1993; Stirke et al. 2014). PEF could be used as a pasteurization alternative for liquid food maintaining temperatures <30–40 °C, thus avoiding the degradation of bioactive compounds (Table 13.3).

Table 13.3 Effect of pulsed electric fields on the stabilization of bioactive compounds

Matrix	About compounds and other implications	Processing conditions			Retention (%)	References
		Electric field strength (kV/cm)	Pulse frequency (Hz)	Temperature (°C)		
Orange juice	Total phenolic concentration, hydroxybenzoic acid, hydroxycinnamic acids, flavonols, flavones, and flavonones	13.82, 17.06, 21.50, 25.26	NR	35–58.20	Phenolic compounds >90 Hydroxybenzoic acid, hydroxycinnamic acids >90 Flavonones >90 Flavonols and flavones >76	Agcam et al. (2014)
Blueberry juice	Ascorbic acid, total phenolics, total anthocyanins, antioxidant capacity	36	NR	<60	Ascorbic acid >96 Total phenolics >100 Total anthocyanins >100 Antioxidant activity >100	Barba et al. (2012)
Orange juice–milk beverage	Ascorbic acid	5, 25, 35, 40	NR	22–62.6	Ascorbic acid >90	Zulueta et al. (2010)
Orange–carrot juice	Ascorbic acid	25, 30, 35, and 40	NR	NR	>89 in all treatments	Torregrosa et al. (2006)
Mixed orange juice and milk beverage	Water-soluble vitamins and ACE inhibitory peptides	15, 25 and 40	NR	<50	Vitamins >92–100 ACE inhibitory peptides >96	Rivas et al. (2007)
Orange juice	Ascorbic acid	25, 30, 35, and 40	NR	<49	81–100	Cortés et al. (2008)
Orange juice	Enzymes and bioactives	23	90	<58	>85 Ascorbic acid >71 dehydroascorbic 100 citric and malic acid	Vervoort et al. (2011)

NR Not reported

Table 13.4 Retention of bioactive compounds after high-pressure processing

Matrix	About compounds and other implications	Processing conditions			References
		Pressure (MPa)	Temperature (°C)	Time (min)	
Grapefruit jam	Antioxidant activity, enzymes	550–700	45–75	2.5–75	Igual et al. (2013)
Plum puree	Polyphenol oxidase, extractability of carotenoids	400, 600	20 and 25	7	González-Cebrino et al. (2012)
Blueberry juice	Antioxidant compounds (ascorbic acid, total phenolics, total anthocyanins) and antioxidant capacity	600	42	5	Barba et al. (2012)
Opuntia macrorhiza juice	Vitamin C, betacyanins, antioxidant activity	600	NR	10	Moussa-Ayoub et al. (2011)
Blood orange juice	Anthocyanins and ascorbic acid	400, 500, 600	~20	15	Torres et al. (2011)
Cold smoked fish	Color, thiobarbituric acid value, trimethylamine nitrogen	220, 250, and 330	3, 7, 15, and 25	5 and 10	Erkan et al. (2011)
Orange juice	Bioactive compounds (carotenoid and flavanone)	400	40	1	Plaza et al. (2011)
Vegetable beverage	High retention of ascorbic acid and no change in phenolic compounds	100, 200, 300, and 400	25–30	2–9	Barba et al. (2010)
Blueberry juice	Total anthocyanins	100–700	40–121	NR	Buckow et al. (2010)
Strawberry and blackberry purees	Phenolic content, anthocyanins, ascorbic acid, and antioxidant activity	400, 500, and 600	20	15	Patras et al. (2009b)
Strawberry	Inactivation of oxidative enzymes, retention of nutritional and physicochemical quality	300, 450, and 600	20–60	2–10	Terefe et al. (2009)
Tomato and carrot purees	Antioxidant activity, bioactive compounds	400–600	20	15	Patras et al. (2009a)
Gazpacho	Carotenoids, antioxidant activity	150–350	60	15	Plaza et al. (2006)

Many applications of PEF have been reported in the literature, and it has been fully demonstrated that the stability of aroma compounds, phytochemicals, and bioactive compounds in fruits and vegetables juice were not significantly affected by PEF processing as listed in Table 13.4 (Aguilar-Rosas et al. 2007; Sampedro et al. 2009; Odriozola-Serrano et al. 2013; Agcam et al. 2014).

Other reports show that there is an increase in phenolic compounds after PEF processing compared with the control. Free phenolic acids are formed by various reactions, such as hydroxylation, methylation, isoprenylation, dimerization, and/or glycosylation, occurring during processing. Such reactions could modify the molecular structure of the moieties of phenolic compounds, thus leading to the formation of a wider range of phenolic compounds (Morales-de La Peña et al. 2011). Cortés et al. (2006) observed that PEF treatments of 35 and 40 kV/cm for 30–240 μs, slightly increased the carotenoid concentration in orange juice, but there was a significant decrease in total carotenoids after application of 30 kV/cm for 100 μs. PEF treatment significantly ($p \leq 0.05$) impacted total phenolic content and total anthocyanin content values in blueberry when an electric field intensity of 1, 3, or 5 kV/cm was applied (Bobinaitė et al. 2014). Wiktor et al. (2015) reported a higher total carotenoid content and total phenolic compounds $\leq$11.34 and 85.8 % compared with untreated plant tissue by the application of 1.85 kV/cm of electric fields and 10 pulses; however, the application of a high number of pulses (50 and 100) caused significant ($p \leq 0.05$) degradation of phenolic compounds.

13.4 High-Pressure Processing

High-pressure processing (HPP) was originally used in the production of ceramics, steel, and super alloys. The inactivation of microorganisms by high pressure has been recognized since the beginning of the twentieth century. Because the pressure is rapidly and uniformly distributed throughout the medium in high-pressure processing for food applications, the inactivation of microorganisms and enzymes occurs evenly in the product.

High-pressure processing is considered a nonthermal technology even though a small increase in temperature and a change in volume take place with an increase of pressure of the system. With the application of pressure in foods in the range of 50 to 700 MPa, it is possible to inactive spoilage microorganisms and enzymes with minimal change of sensory quality and less damage of nutrients in food compared with conventional thermal processing. The mechanism of disruption of microbial cells involves the breakdown of non-covalent bonds and permeabilization of the cell membrane (Ohlsson and Bengtsson 2002).

Many equipment designs have been developed to create a more efficient processes at low cost. For example, processing at 103 MPa and 40–60 °C for 2.5 min improves the eating quality of meat and reduces cooking losses (Ohlsson and Bengtsson 2002).

High-pressure treatment of plant cells at 250 MPa for 10 min caused 99 % pigment release to the medium (Knorr 1993). Other research has found that the concentrations of bioactive compounds, such as antioxidants and carotenoids, did not change, or small changes were present, during processing (Table 13.4). As shown, high pressure between 100 and 400 MPa for 1–5 min 30–60 °C resulted in <10 % vitamin C loss in high-pressure treatments combined with heat at 60 °C. Igual et al. (2013) found no significant difference ($p > 0.05$) in antioxidant capacity in grapefruit jam treated by HPP at 550–700 MPa at 45–75 °C. Barba et al. (2012) observed no significant difference ($p > 0.05$) in ascorbic acid and total phenolics in blueberry juice treated by HPP at 600 MPa for 5 min. Another study showed that a significantly higher concentration of individual carotenoids was recovered after high-pressure processing of orange juice (Plaza et al. 2011). In high-pressure processing of orange juice–milk beverage at 100 MPa for 420 s, there was a significantly greater increase (22 % greater than untreated sample) in total phenolic compounds (Barba et al. 2011). In studies of the refrigerated storage of high-pressure processed (550 MPa, 5 min, and room temperature) blended pepper–orange juice, Xu et al. (2015) found 77.3 % retention of total phenolic content, 90.8 % retention of ascorbic acid, and >80 % retention of antioxidant capacity after 25 days of treatment.

13.5 Cold Plasma

Cold plasma, which is also called the "fourth state of matter," is composed of energetic, reactive gases used to inactivate contaminating microorganisms on food surfaces. The main modes of microbial inactivation are reactive chemical species and ultraviolet light. It is a relatively new nonthermal technology that has shown promising results in the surface decontamination of foods. Cold plasma has potential in the control of microbial populations without affecting physicochemical and sensory attributes of foods. Cold plasma for food processing relies on the application of the plasma directly to the food products or indirectly through packages.

The inactivation of microorganisms by cold plasma is mainly due to the effect of heat, charged particles, electric fields, UV photons, and some reactive species such as atomic oxygen, metastable oxygen molecules, ozone, and hydroxyl radicals, which are commonly found in a gas discharge. Being a surface treatment, cold plasma is particularly suitable to preserve bioactive compounds (Table 13.5).

A 70 % reduction of polyphenol oxidase (PPO) was achieved after 60 s of plasma exposure in the model food systems (Surowsky et al. 2013). Pankaj et al. (2013) found a significant reduction ($p < 0.05$) of tomato peroxidase after plasma treatment by 30, 40, and 50 kV. Enzyme activity was decreased with an increase in voltage and treatment time. A reduction of 4 log cycle was reported by Deng et al. (2006) in almonds treated by cold plasma at 25 kV and 2 kHz reduction of *Salmonella Stanley* and *E. coli* by 3.7 and 3.6 log, respectively, was observed by Niemira and Sites (2008) on apple surfaces using cold plasma generated in a gliding arc.

Table 13.5 Application of cold plasma to preserve bioactive compounds in food

Matrix	About compounds and other implications	Process	Processing conditions			References
			Tension/voltage/power	Pulses/frequency	Time	
Strawberries	Color and firmness	Atmospheric cold plasma	60 kV dielectric barrier discharge (DBD) 40-mm gap	50 Hz	5 min (indirect treatment)	Misra et al. (2014)
Lettuce, carrots, and tomatoes	Color and cell structure	Pressure cold plasma	Needle array from 3.95 kV up to 12.83 kV in argon	60 Hz	30 s to 10 min	Bermúdez-Aguirre et al. (2013)
Model food system	Polyphenol-oxidase (PPO) and peroxidase (POD) inactivation	Cold atmospheric pressure plasma jet	65 V in argon	1.1 MHz	0–360 s	Surowsky et al. (2013)
Corn salad leaves	Photosynthetic activity (quality retention)	Atmospheric pressure plasma jet	10, 20, 30, and 40 W	27.12 MHz	NR	Baier et al. (2013)
Tomato	Peroxidase	Atmospheric pressure cold plasma	In-package at 30, 40, and 50 kV	NR	$\leq$5 min	Pankaj et al. (2013)
RTE meat product (bresaola)	Thiobarbituric acid value and color	Cold atmospheric pressure	15.5, 31, and 62 W inside sealed LLD-PE bags containing 30 % oxygen and 70 % argon	27.8 kHz	2–60 s	Rød et al. (2012)
Fresh pork	Color, pH, fluorescence, and reflectance	Microwave indirect-plasma treatment	1.2 kW; process gas air (microwave setup)	2.45 GHz	2 × 2.5 or 5 × 2 min	Fröhling et al. (2012)
Fresh fruit and vegetable slices	Color parameters, nutritional content	Plasma microjet	Compressed air, 30 mA at 500 V	NR	$\leq$8 × 60 s	Wang et al. (2012)

NR Not reported

Cold plasma has shown some adverse effects on the lipid content of the food that results in the formation by oxidation of hydroxyl acids, keto acids, short-chain fatty acids, and aldehydes, among others. This oxidation, at it is well known, is responsible for "off" flavors and odors. Therefore, food products with high fat content—such as meat, whole milk, etc.—may not be ideal for processing by cold plasma (Critzer et al. 2007). Kovačević et al. (2015) found a significant ($p < 0.05$) increase in the concentration of total anthocyanin content in pomegranate juice after cold-plasma treatment for 3, 5, or 6 min. The application of cold plasma in a field at 70 kV for 15, 30, 45, and 60 s caused no significant changes in pH, color, total phenolic content, and total antioxidant capacity of orange juice (Almeida et al. 2015). Kiwi fruits processed by cold plasma for 10 and 20 min had no significant changes in lightness (L value) compared with untreated fruit, which indicated that cold-plasma processing could result in less-darkened product (Ramazzina et al. 2015). These authors also concluded that there were no significant changes ($p > 0.05$) in antioxidants content and antioxidant activity compared with untreated fruit.

13.6 Combined Technologies

To increase the effectiveness of the reduction of microorganisms and extend of shelf life of food products, new technologies have been combined, among them or with conventional technologies, such as heat processing or the addition of antimicrobial agents. A combination of two or more technologies increases the effectiveness either by synergistic or additive effects. The combination of several processes is more energy efficient, requires lower intensities and shorter treatment times, and less impact on sensory and physicochemical properties compared with conventional heat processes. Ultrasound has been well combined with pressure (manosonication) and temperature (thermosonication). High-pressure processing has given good results in combination with high temperature (known as "pressure-assisted thermal processing" [PATP]) in stabilization of food to make it (in some cases) shelf stable.

13.6.1 Pulsed Electric Fields

Processing with pulsed electric fields combines well with temperature, ultraviolet, and/or pulsed light to enhance the extraction efficiency. Caminiti et al. (2011) reported no effect on the color, odor, and flavor of an apple-and-cranberry juice blend treated either by pulsed electric fields and ultraviolet or by pulsed electric fields and high-intensity light pulses. Both of these combinations successfully retained more monomeric anthocyanins.

13.6.2 High-Pressure Processing

Mild temperature and osmotic dehydration are commonly combined with high-pressure processing. Most of the reports on HPP suggest that this combination increases the retention rate compared with HPP alone. The combination of osmotic dehydration and high-pressure processing (200, 400, and 500 MPa) for 10 min retained Vitamin C content by 98 % in strawberries (Nuñez-Mancilla et al. 2013); the same authors have also reported a decrease in browning index or lightness (L^*), which indicated that fruit treated with HPP and osmotic dehydration are lighter in color compared with fresh samples. The combination of high pressure (300 MPa) and temperature (40 °C) for 6 min had no significant effect on the total polyphenol content of strawberries, whereas treatment at 400 MPa and 40 °C for 6 min caused a 37 % loss of total polyphenols (Terefe et al. 2009). Ultrafiltration of Korla pear juice followed by high-pressure processing resulted in no significant changes ($p > 0.05$) in total phenols, antioxidant capacity, and ascorbic acid compared with ultrafiltration; however, the above-mentioned characteristics were significantly ($p < 0.05$) affected in the combined processing of ultrafiltration with high treatment and short treatment time (Zhao et al. 2016).

13.6.3 Ultrasound

Many foods have been processed by combining ultrasound with temperature or ultrasound and pressure in different ranges. Previous reports have shown an increase in the effectiveness of ultrasound in the reduction of microorganisms by combining it with pressure and heat (Bermúdez-Aguirre et al. 2009; Arroyo et al. 2011; Bermúdez-Aguirre and Barbosa-Cánovas 2012). In addition, the combination of ultrasound with temperature and pressure also increased the lethality against spores and heat-resistant microorganisms such as *Bacillus subtilis* because a greater imbalance and penetration of cell membranes took place.

If food is treated under a combined ultrasound-and-thermal process, this process is known as "*thermosonication*;" if it is combined with pressure, it is called "*manosonication*;" if all three processes are combined, it is called "*manothermosonication"* (Chemat and Khan 2011).

The thermosonication and manothermosonication processes have been focused on microbial or enzymatic inactivation (Arroyo et al. 2011; Bermúdez-Aguirre and Barbosa-Cánovas 2012; Cregenzán-Alberti et al. 2014). Only a few studies describe the effects of thermosonication and manothermosonication on bioactive compounds (Table 13.3). As listed in Table 13.6, thermosonication and manothermosonication exerted a high percentage (>90 % in almost all cases) in the retention of bioactive compounds compared with conventional thermal processing such as boiling, pressure cooking, baking, and frying, in which loss of nutritional and antioxidant compounds is approximately 6–60 % (Jiménez-Monreal et al. 2009).

Critical pressure and temperature levels for achieving the maximum synergistic effect should be chosen in order to provide high reduction of microorganism and

Table 13.6 Retention of bioactive compounds by sonication and combined technologies

Matrix	Process	Processing conditions			Bioactive compound		Retention (%)	References
		Frequency, power, amplitude, pressure	Temperature (°C)	Time				
Apple juice	Thermo-sonication using ultrasound in a bath	25 kHz, 70 % power (500 W)	20, 40 and 60	30 min	Ascorbic acid		>90	Abid et al. (2013)
					Total phenolics		>85	
Apple juice	Combination of pulsed light and thermo-sonication	24 Hz, 100 % Power (400 W)	40–50	171–300 s	Antioxidant activity (TEAC)		93 and 98	Muñoz et al. (2012)
					Color	*L*	94.4–98	
						a	74.8–81.1	
						b	89.6–94.5	
Watermelon juice	Thermo-sonication	20 kHz	25–45	2–10 min (5 s on and 5 s off)	Color		NR	Rawson et al. (2011)
					Lycopene content		46.35–106.68	
					Phenolic content		41.46–83.23	
					Ascorbic acid		73.7–98.6	
					Vitamin C		95 %	
Watercress (*Nasturtium officinale*)	Thermo-sonication	20 kHz 50 % of power (125 W)	86 and 92	2 and 30 s	Vitamin C		~94 %	Cruz et al. (2011)
Watercress (*Nasturtium officinale*)	Thermo-sonication	20 kHz 50 % of power (125 W)	82.5–92.5	NR	Vitamin C		62.93–86.81	Cruz et al. (2008)
					Total phenolics		94–95	

NR not reported

inhibition of enzymes, but high retention of bioactive compounds is also important. Cavitation phenomena can be decreased by pressure and heat: As temperature increases, vapor pressure increases, thus producing a reduction in energy release during bubble implosion. In contrast, if there is an increase in pressure, ultrasound waves are unable to overcome the cohesive forces of the liquid molecules (Condón et al. 2004; Sango et al. 2014).

Sometimes it is not possible to compare bioactive-compound retention after thermosonication and manothermosonication processes due to variations in power, time, temperature, and food matrixes. Some researchers have reported that the effect on bioactive compounds in food during thermosonication and manothermosonication, as well as when they are combined with other technologies, might not only be due to pressure, heat, and/or ultrasound treatments (Palgan et al. 2011; Muñoz et al. 2012; Halpin et al. 2013).

13.7 Final Remarks

Nonthermal technologies for the stabilization of bioactive compounds represents a promising application that is currently being explored. It can be used to deliver microbiologically safe products with stabilization of intracellular constituents and preservation of sensory attributes of products at optimum levels. Some nonthermal technologies are still in their early stages of development, but others have been implemented at the industrial-scale for commercial uses. Foods rich in bioactive compounds can be processed using selected nonthermal technologies and a selected combination for stabilization and extraction. Nonthermal technologies have also shown good results in reducing enzyme activity and increase of the shelf life of products without significantly altering their physicochemical properties. Many studies have clearly shown that there are no significant changes in the quantity of bioactive compounds present in the food and that in some cases there is even an increase in those compounds; furthermore, it has been shown that degradation is not as common as in conventional thermal processing.

References

Abbas S, Bashari M, Akhtar W, Li WW, Zhang X (2014) Process optimization of ultrasound-assisted curcumin nanoemulsions stabilized by OSA-modified starch. Ultrason Sonochem 21(4):1265–1274

Abbas S, Hayat K, Karangwa E, Bashari M, Zhang X (2013) An overview of ultrasound-assisted food-grade nanoemulsions. Food Eng Rev 5(3):139–157

Abid M, Jabbar S, Wu T, Hashim MM, Hu B, Lei S, Zhang X, Zeng X (2013) Effects of ultrasound on different quality parameters of apple juice. Ultrason Sonochem 20:1182–1187

Agcam E, Akyıldız A, Evrendilek GA (2014) Comparison of phenolic compounds of orange juice processed by pulsed electric fields (PEF) and conventional thermal pasteurization. Food Chem 143:354–361

Aguilar-Rosas SF, Ballinas-Casarrubias ML, Nevarez-Moorillon GV, Martín-Belloso O, Ortega-Rivas E (2007) Thermal and pulsed electric fields pasteurization of apple juice: effects on physicochemical properties and flavour compounds. J Food Eng 83(1):41–46

Almeida FDL, Cavalcante RS, Cullen PJ, Frias JM, Bourke P, Fernandes FA, Rodrigues S (2015) Effects of atmospheric cold plasma and ozone on prebiotic orange juice. Innov Food Sci Emerg Technol 32:127–135

Anese M, Mirolo G, Beraldo P, Lippe G (2013) Effect of ultrasound treatments of tomato pulp on microstructure and lycopene in vitro bioaccessibility. Food Chem 136(2):458–463

Anton N, Benoit J, Saulnier P (2008) Design and production of nanoparticles formulated from nano-emulsion templates: a review. J Controlled Release 128:185–199

Arroyo C, Cebrián G, Pagán R, Condón S (2011) Inactivation of Cronobacter *sakazakii* by manothermosonication in buffer and milk. Int J Food Microbiol 151(1):21–28

Ashokkumar M (2011) The characterization of acoustic cavitation bubbles: an overview. Ultrason Sonochem 18(4):864–872

Baier M, Foerster J, Schnabel U, Knorr D, Ehlbeck J, Herppich WB, Schlüter O (2013) Direct non-thermal plasma treatment for the sanitation of fresh corn salad leaves: evaluation of physical and physiological effects and antimicrobial efficacy. Postharvest Biol Technol 84:81–87

Barba FJ, Cortés C, Esteve MJ, Frígola A (2011) Study of antioxidant capacity and quality parameters in an orange juice–milk beverage after high-pressure processing treatment. Food Bioprocess Technol 5(6):2222–2232

Barba FJ, Esteve MJ, Frígola A (2010) Ascorbic acid is the only bioactive that is better preserved by high hydrostatic pressure than by thermal treatment of a vegetable beverage. J Agric Food Chem 58:10070–10075

Barba FJ, Jäger H, Meneses N, Esteve MJ, Frígola A, Knorr D (2012) Evaluation of quality changes of blueberry juice during refrigerated storage after high-pressure and pulsed electric fields processing. Innov Food Sci Emerg Technol 14:18–24

Bermúdez-Aguirre D, Barbosa-Cánovas GV (2012) Inactivation of *Saccharomyces cerevisiae* in pineapple, grape and cranberry juices under pulsed and continuous thermo-sonication treatments. J Food Eng 108(3):383–392

Bermúdez-Aguirre D, Corradini MG, Mawson R, Barbosa-Cánovas GV (2009) Modeling the inactivation of *Listeria innocua* in raw whole milk treated under thermo-sonication. Innov Food Sci Emerg Technol 10(2):172–178

Bermúdez-Aguirre D, Mobbs T, Barbosa-Cánovas GV (2011) Ultrasound applications in food processing. In: Feng H, Barbosa-Cánovas GV, Weiss J (eds) Ultrasound technologies for food and bioprocessing. Springer, New York, pp 65–105

Bermúdez-Aguirre D, Wemlinger E, Pedrow P, Barbosa-Cánovas G, Garcia-Perez M (2013) Effect of atmospheric pressure cold plasma (APCP) on the inactivation of *Escherichia coli* in fresh produce. Food Control 34(1):149–157

Bhat R, Kamaruddin NSBC, Min-Tze L, Karim AA (2011) Sonication improves kasturi lime (*Citrus microcarpa*) juice quality. Ultrason Sonochem 18(6):1295–1300

Bobinaitė R, Pataro G, Lamanauskas N, Šatkauskas S, Viškelis P, Ferrari G (2014) Application of pulsed electric field in the production of juice and extraction of bioactive compounds from blueberry fruits and their by-products. J Food Sci Technol 52:1–8

Buckow R, Kastell A, Terefe NS, Versteeg C (2010) Pressure and temperature effects on degradation kinetics and storage stability of total anthocyanins in blueberry juice. J Agric Food Chem 58:10076–10084

Cameron M, McMaster LD, Britz TJ (2008) Electron microscopic analysis of dairy microbes inactivated by ultrasound. Ultrason Sonochem 15(6):960–964

Caminiti IM, Noci F, Muñoz A, Whyte P, Morgan DJ, Cronin DA, Lyng JG (2011) Impact of selected combinations of non-thermal processing technologies on the quality of an apple and cranberry juice blend. Food Chem 124(4):1387–1392

Castro AJ, Barbosa-Cánovas GV, Swanson BG (1993) Microbial inactivation of foods by pulsed electric fields. J Food Process Preserv 17(1):47–73

Chandrapala J, Oliver C, Kentish S, Ashokkumar M (2012) Ultrasonics in food processing. Ultrason Sonochem 19(5):975–983

Chemat F, Khan MK (2011) Applications of ultrasound in food technology: processing, preservation and extraction. Ultrason Sonochem 18(4):813–835

Condón S, Raso J, Pagán R (2004) Microbial inactivation by ultrasound. In: Barbosa-Cánovas GV, Tapia MS, Cano MP (eds) Novel food processing technologies. CRC Press, Boca Raton, pp 423–442

Cortés C, Esteve MJ, Frígola A (2008) Effect of refrigerated storage on ascorbic acid content of orange juice treated by pulsed electric fields and thermal pasteurization. Eur Food Res Technol 227:629–635

Cortés C, Torregrossa F, Esteve MJ, Frígola A (2006) Carotenoid profile modification during refrigerated storage in untreated and pasteurized orange juice and orange juice treated with high-intensity pulsed electric fields. J Agric Food Chem 54:6247–6254

Cregenzán-Alberti O, Halpin RM, Whyte P, Lyng J, Noci F (2014) Suitability of ccRSM as a tool to predict inactivation and its kinetics for *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas fluorescens* in homogenized milk treated by manothermosonication (MTS). Food Control 39:41–48

Critzer FJ, Kelly-Wintenberg K, South SL, Golden DA (2007) Atmospheric plasma inactivation of foodborne pathogens on fresh produce surfaces. J Food Prot 70(10):2290–2296

Cruz RM, Vieira MC, Fonseca SC, Silva CL (2011) Impact of thermal blanching and thermosonication treatments on watercress (*Nasturtium officinale*) quality: thermosonication process optimisation and microstructure evaluation. Food Bioprocess Technol 4(7):1197–1204

Cruz RM, Vieira MC, Silva CL (2008) Effect of heat and thermosonication treatments on watercress (*Nasturtium officinale*) vitamin C degradation kinetics. Innov Food Sci Emerg Technol 9(4):483–488

Demirdöven A, Baysal T (2008) The use of ultrasound and combined technologies in food preservation. Food Rev Int 25(1):1–11

Deng X, Shi J, Kong MG (2006) Physical mechanisms of inactivation of *Bacillus subtilis* spores using cold atmospheric plasmas. IEEE Trans Plasma Sci 34(4):1310–1316

Erkan N, Üretener G, Alpas H, Selçuk A, Özden Ö, Buzrul S (2011) The effect of different high pressure conditions on the quality and shelf life of cold smoked fish. Innov Food Sci Emerg Technol 14:18–24

Feng H, Barbosa-Cánovas GV, Weiss J (2011) Ultrasound technologies for food and bioprocessing. Springer, New York

Fröhling A, Durek J, Schnabel U, Ehlbeck J, Bolling J, Schlüter O (2012) Indirect plasma treatment of fresh pork: decontamination efficiency and effects on quality attributes. Innov Food Sci Emerg Technol 16(2012):381–390

Gharibzahedi SMT, Razavi SH, Mousavi SM (2013) Ultrasound-assisted formation of the canthaxanthin emulsions stabilized by arabic and xanthan gums. Carbohydr Polym 96(1):21–30

Ghosh V, Mukherjee A, Chandrasekaran N (2013a) Ultrasonic emulsification of food-grade nanoemulsion formulation and evaluation of its bactericidal activity. Ultrason Sonochem 20 (1):338–344

Ghosh V, Saranya S, Mukherjee A, Chandrasekaran N (2013b) Cinnamon oil nanoemulsion formulation by ultrasonic emulsification: investigation of its bactericidal activity. J Nanosci Nanotechnol 13(1):114–122

González-Cebrino F, García-Parra J, Contador R, Tabla R, Ramírez R (2012) Effect of high-pressure processing and thermal treatment on quality attributes and nutritional compounds of "Songold" plum puree. J Food Sci 77(8):C866–C873

Halpin RM, Cregenzán-Alberti O, Whyte P, Lyng JG, Noci F (2013) Combined treatment with mild heat, manothermosonication and pulsed electric fields reduces microbial growth in milk. Food Control 34(2):364–371

Igual M, Sampedro F, Martínez-Navarrete N, Fan X (2013) Combined osmodehydration and high pressure processing on the enzyme stability and antioxidant capacity of a grapefruit jam. J Food Eng 114:514–521

Jiménez-Monreal AM, García-Diz L, Martínez-Tomé M, Mariscal M, Murcia MA (2009) Influence of cooking methods on antioxidant activity of vegetables. J Food Sci 74(3):H97–H103

Jin TZ, Guo M, Zhang HQ (2015) Upscaling from benchtop processing to industrial scale production: more factors to be considered for pulsed electric field food processing. J Food Eng 146:72–80

Keenan DF, Tiwari BK, Patras A, Gormley R, Butler F, Brunton NP (2012) Effect of sonication on the bioactive, quality and rheological characteristics of fruit smoothies. Int J Food Sci Technol 47:827–836

Knorr D (1993) Effect of high hydrostatic pressure on food safety and quality. Food Technol 47 (6):156–161

Komes D, Belščak-Cvitanović A, Domitran Z, Opalić M (2013) Content of saccharides, antioxidant and sensory properties of pear cultivar "Abate Fete" affected by ultrasound pre-treatment and air drying duration. J Food Nutr Res 52:239–250

Kovačević DB, Putnik P, Dragović-Uzelac V, Pedisić S, Jambrak AR, Herceg Z (2015) Effects of cold atmospheric gas phase plasma on anthocyanins and color in pomegranate juice. Food Chem 190:317–323

Leighton TG (1998) The principles of cavitation. In: Povey MJW, Mason TJ (eds) Ultrasound in food processing. Blackie Academic and Professional, London, pp 151–182

Louisnard O, González-García J (2011) Acoustic cavitation. In: Feng H, Barbosa-Cánovas GV, Weiss J (eds) Ultrasound technologies for food and bioprocessing. Springer, New York, pp 13–64

Marchesini G, Balzan S, Montemurro F, Fasolato L, Andrighetto I, Segato S, Novelli E (2012) Effect of ultrasound alone or ultrasound coupled with CO_2 on the chemical composition, cheese-making properties and sensory traits of raw milk. Innov Food Sci Emerg Technol 16:391–397

McClements DJ (1995) Advances in the application of ultrasound in food analysis and processing. Trends Food Sci Technol 6(9):293–299

Misra NN, Patil S, Moiseev T, Bourke P, Mosnier JP, Keener KM, Cullen PJ (2014) In-package atmospheric pressure, cold plasma treatment of strawberries. J Food Eng 125(1):131–138

Mongenot N, Charrier S, Chalier P (2000) Effect of ultrasound emulsification on cheese aroma encapsulation by carbohydrates. J Agric Food Chem 48(3):861–867

Moody A, Marx G, Swanson BG, Bermúdez-Aguirre D (2014) A comprehensive study on the inactivation of *Escherichia coli* under nonthermal technologies: high hydrostatic pressure, pulsed electric fields and ultrasound. Food Control 37:305–314

Morales-de La Peña M, Salvia-Trujillo L, Rojas-Graü MA, Martín-Belloso O (2011) Changes on phenolic and carotenoid composition of high intensity pulsed electric field and thermally treated fruit juice–soymilk beverages during refrigerated storage. Food Chem 129(3):982–990

Moussa-Ayoub TE, Jaeger H, Knorr D, El-Samahy S, Rohn S, Kroh LW (2011) Impacts of traditional and innovative technologies on some characteristics and bioactive compounds of *Opuntia macrorhiza* juice. Procedia Food Sci 1:1410–1416

Muñoz A, Caminiti IM, Palgan I, Pataro G, Noci F, Morgan DJ, Lyng JG et al (2012) Effects on *Escherichia coli* inactivation and quality attributes in apple juice treated by combinations of pulsed light and thermosonication. Food Res Int 45(1):299–305

Niemira BA, Sites J (2008) Cold plasma inactivates *Salmonella* Stanley and *Escherichia coli* O157:H7 inoculated on golden delicious apples. J Food Prot 71(7):1357–1365

Nuñez-Mancilla Y, Pérez-Won M, Uribe E, Vega-Gálvez A, Di Scala K (2013) Osmotic dehydration under high hydrostatic pressure: effects on antioxidant activity, total phenolics compounds, vitamin C and colour of strawberry (*Fragaria vesca*). LWT-Food Sci Technol 52 (2):151–156

Odriozola-Serrano I, Aguilo-Aguayo I, Soliva-Fortuny R, Martin-Belloso O (2013) Pulsed electric fields processing effects on quality and health-related constituents of plant-based foods. Trends Food Sci Technol 29(2):98–107

Ohlsson T, Bengtsson N (2002) Minimal processing of foods with non-thermal methods. In: Ohlsson T, Bengtsson N (eds) Minimal processing technologies in the food industry. Woodhead Publishing, Cambridge, pp 34–60

Palgan I, Caminiti IM, Muñoz A, Noci F, Whyte P, Morgan DJ, Lyng JG et al (2011) Combined effect of selected non-thermal technologies on *Escherichia coli* and *Pichia fermentans* inactivation in an apple and cranberry juice blend and on product shelf life. Int J Food Microbiol 151(1):1–6

Pankaj SK, Misra NN, Cullen P (2013) Kinetics of tomato peroxidase inactivation by atmospheric pressure cold plasma based on dielectric barrier discharge. Innov Food Sci Emerg Technol 19:153–157

Patras A, Brunton N, Da Pieve S, Butler F, Downey G (2009a) Effect of thermal and high pressure processing on antioxidant activity and instrumental colour of tomato and carrot purées. Innov Food Sci Emerg Technol 10:16–22

Patras A, Brunton NP, Da Pieve S, Butler F (2009b) Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. Innov Food Sci Emerg Technol 10:308–313

Plaza L, Sánchez-Moreno C, DeAncos B, Elez-Martínez P, Martín-Belloso O, Cano MP (2011) Carotenoid and flavanone content during refrigerated storage of orange juice processed by high-pressure, pulsed electric fields and low pasteurization. LWT-Food Sci Technol 44 (4):834–839

Plaza L, Sánchez-Moreno C, De Ancos B, Cano MP (2006) Carotenoid content and antioxidant capacity of Mediterranean vegetable soup (*gazpacho*) treated by high-pressure/temperature during refrigerated storage. Eur Food Res Technol 223(2):210–215

Ramazzina I, Berardinelli A, Rizzi F, Tappi S, Ragni L, Sacchetti G, Rocculi P (2015) Effect of cold plasma treatment on physico-chemical parameters and antioxidant activity of minimally processed kiwifruit. Postharvest Biol Technol 107:55–65

Rawson A, Tiwari BK, Patras A, Brunton N, Brennan C, Cullen PJ, O'Donnell C (2011) Effect of thermosonication on bioactive compounds in watermelon juice. Food Res Int 44(5):1168–1173

Rivas A, Rodrigo D, Company B, Sampedro F, Rodrigo M (2007) Effects of pulsed electric fields on water-soluble vitamins and ACE inhibitory peptides added to a mixed orange juice and milk beverage. Food Chem 104(4):1550–1559

Rød SK, Hansen F, Leipold F, Knøchel S (2012) Cold atmospheric pressure plasma treatment of ready-to-eat meat: inactivation of *Listeria innocua* and changes in product quality. Food Microbiol 30(1):233–238

Sales JM, Resurreccion AVA (2009) Maximising resveratrol and piceid contents in UV and ultrasound treated peanuts. Food Chem 117:674–680

Sales JM, Resurreccion AVA (2010a) Maximizing phenolics, antioxidants and sensory acceptance of UV and ultrasound-treated peanuts. LWT-Food Sci Technol 43:1058–1066

Sales JM, Resurreccion AVA (2010b) Phenolic profile, antioxidants, and sensory acceptance of bioactive-enhanced peanuts using ultrasound and UV. Food Chem 122:795–803

Sampedro F, Geveke DJ, Fan X, Rodrigo D, Zhang QH (2009) Shelf-life study of an orange juice–milk based beverage after PEF and thermal processing. J Food Sci 74(2):S107–S112

Sango DM, Abela D, McElhatton A, Valdramidis VP (2014) Assisted ultrasound applications for the production of safe foods. J Appl Microbiol 116(5):1067–1083

Saranya S, Chandrasekaran N, Mukherjee A (2012) Antibacterial activity of eucalyptus oil nanoemulsion against *Proteus mirabilis*. Int J Pharm Pharm Sci 4(3):668–671

Shanmugam A, Ashokkumar M (2014) Ultrasonic preparation of stable flax seed oil emulsions in dairy systems: physicochemical characterization. Food Hydrocolloids 39:151–162

Sobrino-López A, Martín-Belloso O (2010) Review: potential of high-intensity pulsed electric field technology for milk processing. Food Eng Rev 2(1):17–27

Soliva-Fortuny R, Balasa A, Knorr D, Martín-Belloso O (2009) Effects of pulsed electric fields on bioactive compounds in foods: a review. Trends Food Sci Technol 20(11):544–556

Soria AC, Villamiel M (2010) Effect of ultrasound on the technological properties and bioactivity of food: a review. Trends Food Sci Technol 21(7):323–331

Stirke A, Zimkus A, Ramanaviciene A, Balevicius S, Zurauskiene N, Saulis G, Ramanavicius A et al (2014) Electric field-induced effects on yeast cell wall permeabilization. Bioelectromagnetics 35(2):136–144

Surowsky B, Fischer A, Schlüter O, Knorr D (2013) Cold plasma effects on enzyme activity in a model food system. Food Microbiol 30:233–238

Terefe NS, Matthies K, Simons L, Versteeg C (2009) Combined high pressure-mild temperature processing for optimal retention of physical and nutritional quality of strawberries (*Fragaria* × *ananassa*). Innov Food Sci Emerg Technol 10(3):297–307

Torregrosa F, Esteve MJ, Frígola A, Cortés C (2006) Ascorbic acid stability during refrigerated storage of orange–carrot juice treated by high pulsed electric field and comparison with pasteurized juice. J Food Eng 73:339–345

Torres B, Tiwari BK, Patras A, Cullen PJ, Brunton N, O'Donnell CP (2011) Stability of anthocyanins and ascorbic acid of high pressure processed blood orange juice during storage. Innov Food Sci Emerg Technol 12:93–97

Vervoort L, Van der Plancken I, Grauwet T, Timmermans RAH, Mastwijk HC, Matser AM, Hendrickx ME, Van Loey A (2011) Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice. Part II: impact on specific chemical and biochemical quality parameters. Innov Food Sci Emerg Technol 12:466–477

Wang RX, Nian WF, Wu HY, Feng HQ, Zhang K, Zhang J, Fang J et al (2012) Atmospheric-pressure cold plasma treatment of contaminated fresh fruit and vegetable slices: inactivation and physiochemical properties evaluation. Eur Phys J D-At Mol Opt Plasma Phys 66(10):1–7

Wiktor A, Sledz M, Nowacka M, Rybak K, Chudoba T, Lojkowski W, Witrowa-Rajchert D (2015) The impact of pulsed electric field treatment on selected bioactive compound content and color of plant tissue. Innovative Food Sci Emerg Technol 30:69–78

Xu Z, Lin T, Wang Y, Liao X (2015) Quality assurance in pepper and orange juice blend treated by high pressure processing and high temperature short time. Innov Food Sci Emerg Technol 31:28–36

Zhang Q, Barbosa-Cánovas GV, Swanson BG (1995) Engineering aspects of pulsed electric field pasteurization. J Food Eng 25(2):261–281

Zhao L, Wang Y, Hu X, Sun Z, Liao X (2016) Korla pear juice treated by ultrafiltration followed by high pressure processing or high temperature short time. LWT-Food Sci Technol 65:283–289

Zulueta A, Esteve MJ, Frígola A (2010) Ascorbic acid in orange juice–milk beverage treated by high intensity pulsed electric fields and its stability during storage. Innov Food Sci Emerg Technol 11(1):84–90

Chapter 14
Chemical Stability: Browning and Oxidation

Naritchaya Potes, Aaron S.L. Lim and Yrjö H. Roos

Abstract A number of food components are sensitive to deteriorative reactions, such as nonenzymatic browning and oxidation, during food storage. Food components, i.e., carbohydrates, lipids, proteins and water undergo changes due to the surrounding atmosphere, the presence of minor components and catalysts, and variations in local reactant concentrations resulting from changes in temperature, water migration, and the state of the components. Bioactive proteins and peptides may participate in nonenzymatic browning and oxidation reactions. Oil-soluble bioactive components, for example carotenoids, need protection against oxidation. Water content, and often the physical state of components as well as food structure, may have a significant impact on bioactive stability during food manufacturing and storage.

Keywords Bioactives · Carotenoids · Glass transition · Maillard reaction · Nonenzymatic browning · Proteins · Oxidation · Vitamins · Water

14.1 Introduction

Typical reactions of components in biological and food materials (proteins, lipids, and carbohydrates) such as nonenzymatic browning reaction (NEB) or or glycation (nonenzymatic glycosylation), and oxidation, result in changes in chemical, physical, thermal, and mechanical properties. In food and pharmaceutical materials, such component interactions may affect productivity, product quality, stability, and shelf life. Browning reactions, lipid and protein oxidation in high-solids systems containing proteins, lipids, and carbohydrate components, lead to changes in hydrophilic and hydrophobic interactions of components with consequent deterioration of physicochemical and nutritional quality. Typical examples of materials exhibiting

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such deterioration during storage include confectionary products, dairy foods (infant formulae, ice cream, and hard cheese products), meat products, formulated foods such as high-protein bars, sport foods, or supplements, and pharmaceutical formulations. High-solids foods, and particularly high-protein formulations, are widely studied because of their relevance as models of industrial formulated foods, cosmetics, medicinal foods, and pharmaceuticals. The components of edible high-solids and high-protein systems generally consist of carbohydrates (e.g., fibre, starch, and modified starch, maltodextrins, sugars, gum arabic, and glycerol), proteins {e.g., various milk, meat, egg, plant proteins}, lipids including lipophilic bioactives, humectants or lubricants, and emulsifying agents. Interactions and chemical reactions of components often depend on water activity (a_w) and water content (Labuza et al. 1972; Labuza and Saltmarch 1981; Bell 1996; Acevedo et al. 2008), matrix or structure (Buera and Karel 1995; Lievonen et al. 1998), pH (Hodge 1953; Wolfrom et al. 1974; Ashoor and Zent 1984; Ajandouz and Puigserver 1999; Richards and Hultin 2000; Ajandouz et al. 2001), temperature (Labuza and Saltmarch 1981; Bell 1996; Roos et al. 1996a; Lievonen et al. 1998; Schebor et al. 1999; Akhtar and Dickinson 2007), time under processing and storage (Labuza and Saltmarch 1981; Kato et al. 1989), type and concentration of reactants (Wolfrom et al. 1974; Ashoor and Zent 1984; Kato et al. 1989; Kwak and Lim 2004), the presence of other components such as acids and bases (Ortwerth and Olesen 1988), buffers (Bell 1997), and salts (Kwak and Lim 2004), and environmental conditions [e.g., light and oxygen (Ahmed et al. 1986)]. Non-crystalline amorphous solids often show structural changes (collapse, shrinkage, deformation, crystallization, stickiness, softening, or hardening) during dehydration processes and storage at temperatures above the relevant glass transition temperature (T_g). The physical state or structure of the components also affects rates of chemical reactions. Collapse and deformation of the matrix (Buera and Karel 1995), crystallization of sugars (Shimada et al. 1991; Roos et al. 1996a; Burin et al. 2004; Buera et al. 2005; Acevedo et al. 2006; Drusch et al. 2006), phase separation and diffusion or mobility of reactants in the matrix (Karmas et al. 1992; Roos et al. 1996a; Lievonen et al. 1998; Schebor et al. 1999) were shown to increase rates of nonenzymatic browning and lipid oxidation during storage above the T_g. The T_g and water content of the systems govern molecular mobility and diffusion that relate to physical stability and occurrence of chemical reactions in foods during storage (Slade et al. 1991; Roos 1995a, b). Acevedo et al. (2006) found that the collapsed matrices of freeze-dried models [polyvinylpyrrolidone (PVP) systems] behaved like fluid (liquid-like structure with low density), not crystallized, after storage at 70 °C above 33 % relative humidity (RH) and they showed a decrease of nonenzymatic browning rate with increasing RH due to the dilution of the reactant at constant reactant mass. They also found that the maximum NEB rate occurred at temperatures above and close to T_g, before the collapse of the sample, and in the supercooled region, when sugar crystallization was favoured. The presence of other insoluble components in the systems affecting structure, collapse, and crystallization could modify and shift the browning rate towards high RH in the supercooled (rubbery) state, at least 60 °C above the T_g of the system.

The present chapter reviews chemical reactions and component interactions (carbohydrate, protein, and lipid), and their impact on properties of high-solids systems. This information will be useful for understanding the interactions between food components (e.g., carbohydrate–carbohydrate, protein–oil, protein–sugar, and protein–oil–sugar) and protein behaviour (hydration, denaturation, and aggregation) in dehydrated foods (high-solids systems), pharmaceutical materials, and formulations with bioactive components under various conditions during processing and storage. Information presented here has wide areas of application, as similar findings have been used to explain physical, physicochemical, and thermal properties and structural changes in proteins that are affected by chemical reactions (lipid and protein oxidation, and Maillard reaction) in foods (e.g., confectionary products, hard cheese products, infant formulae), dairy powders and ingredients, high-protein snack bars and high-protein foods (including sausages, dried-meat, frozen fish, and meat products), supplements (reconstituted and food-mixed powder for energy fuel beverages and muscle building) and pharmaceutical products during thermal storage, and in vivo to address chronic diseases (e.g., diabetes, renal failure, and rheumatoid arthritis), age-related diseases (e.g., atherosclerosis and coronary artery diseases), neurodegenerative diseases (e.g., Alzheimer's, Huntington's, and Parkinson's disease), and uremia. In summary, the outcomes of our studies can be applied in various areas such as food, medicine, and nutritional and pharmaceutical sciences to design and develop high-solids formulations suitable for food and drug products and bioactive delivery systems, and to improve quality and delivery of nutrients.

14.2 Chemical Reactions in High-Solids Systems

14.2.1 Maillard Reaction

The Maillard reaction (nonenzymatic browning reaction, NEB) results from reactions of amines, amino acids, peptides, and proteins with carbonyl groups of reducing sugars (aldehydes and ketones), and leads to the formation of brown pigments or coloured polymers called melanoidins (Fig. 14.1) (Hodge 1953). This chemical reaction is the most common type of browning reaction in foods heated during their processing and storage, and is relevant to human health as the reaction occurs in vivo during ageing (Hodge 1953; Monnier 1990). Maximum browning rates of the Maillard reaction in foods occur at a_w between 0.60 and 0.90 (Labuza and Dugan 1971). The Maillard reaction can be divided into three stages: initial (sugar-amine condensation to form a Schiff base, and Amadori rearrangement), intermediate (dehydration and fragmentation of sugar and degradation of amino acid), and final (aldol condensation and aldehyde-amine polymerization), as shown in Fig. 14.1.

The first reaction of aldose or ketose sugar with amine groups in proteins or other molecules leads to *N*-substituted glycosylamine or *N*-substituted ketosylamine, respectively. This first step is followed by the formation of an Amadori

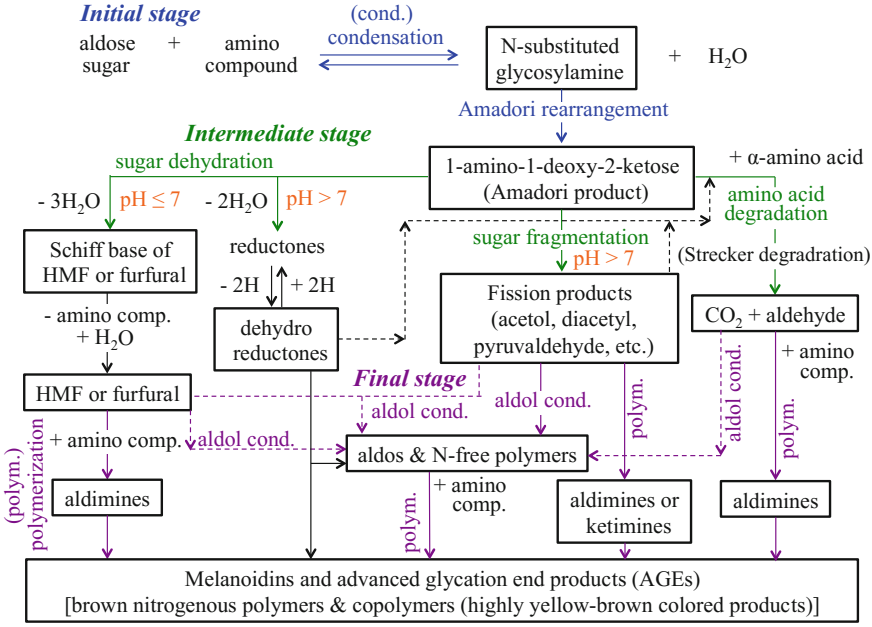

Fig. 14.1 Schematic of Maillard reaction pathways in foods (*HMF* hydroxymethylfurfural) [Adapted with permission from Hodge (1953). Copyright 1953 American Chemical Society]

(1-amino-1-deoxy-2-ketose) or Heyns rearrangement product (2-amino-2-deoxyaldoses), respectively (Hodge 1953; Monnier 1990; Dills 1993). The Amadori or Heyns rearrangement step is generally referred to as "glycation", although various terms such as glucation, fructation, and ribation are used to indicate a specific type of the sugar component of the reaction, i.e., glucose, fructose, and ribose, respectively (Monnier 1990). Formation of Amadori products (colourless compounds) is followed by formation of an increased quantity of the unsaturated carbonyl compounds produced by sugar dehydration (furfural formation) and sugar fragmentation (degradation of sugar) reactions, and Strecker degradation (degradation of amino acid) (Hodge 1953; Nursten 1981). The degradation of sugars and amino acids is a major source of low molecular weight carbonyl compounds (Wells-Knecht et al. 1995; Anderson et al. 1997). The carbonyl compounds can be precursors of both oxidative advanced glycation end products (AGEs) [N^{ϵ}-(carboxymethyl)lysine (CML) and pentosidine] and non-oxidative AGEs derived from 3-deoxyglucosone (deoxyglucosone-lysine dimer) and methylglyoxal (methylglyoxal-lysine dimer) (Reddy et al. 1995; Liggins and Furth 1997; Miyata et al. 1998; Singh et al. 2001).

Reducing sugars can react non-enzymatically with lipids and nucleic acids to form Schiff bases and Amadori products, and subsequently AGEs (irreversible chemical modification and covalent cross-linking of proteins, and browning) (Makita et al. 1991; Dyer et al. 1993; Wells-Knecht et al. 1995) typical of the later stages of the Maillard reaction pathways (Singh et al. 2001). If oxidation

accompanies glycation or Amadori compound formation, then the products formed are known as glycoxidation products—for example, N^{ε}-(carboxymethyl)lysine (CML) (Ahmed et al. 1986; Dyer et al. 1993) and pentosidine (cross-linking between arginine and lysine residues) (Sell and Monnier 1989; Grandhee and Monnier 1991; Dyer et al. 1991, 1993). The AGE in the non-oxidative pathway is pyrraline (Hayase et al. 1989). The lipid peroxidation forms glyoxal that can be formed in the Maillard reaction as the AGE (Thornalley et al. 1999; Singh et al. 2001) (Fig. 14.2). The AGE formation pathways in lived proteins (e.g., connective tissue, membrane, skin collagen) in vivo are shown in Fig. 14.2, which are similar to those in the Maillard reaction in foods (Fig. 14.1). The structure of AGEs is shown in Fig. 14.3. The formation and accumulation of AGEs in vivo is involved in the development of complicated chronic diseases such as diabetes (McCance et al. 1993; Beisswenger et al. 1993), age-related diseases such as atherosclerosis (Lyons 1993; Colaco and Roser 1994; Horiuchi 1996), and neurodegenerative diseases (Smith et al. 1994; Sasaki et al. 1998). Many studies have also used the CML and pentosidine as biochemical markers for assessing advanced glycation reactions in cumulative damage to proteins (Dyer et al. 1991), diabetes (Ahmed et al. 1986; Tenada and Monnier 1994) and diabetic vascular complications such as diabetic nephropathy (Hirata and Kubo 2004) and diabetic retinopathy (Salman et al. 2009; Ghanem et al. 2011), and uremia (Tenada and Monnier 1994; Degenhardt et al. 1997).

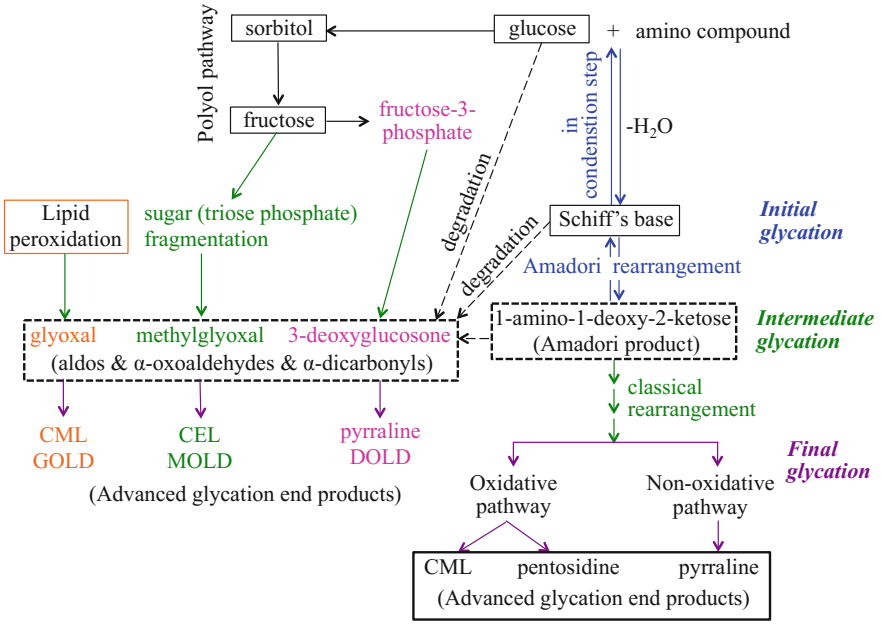

Fig. 14.2 The advanced glycation end products (AGEs) formation pathways in vivo (Singh et al. 2001) (With permission from Springer). The AGEs are N^{ε}-(carboxymethyl)lysine (CML), pentosidine, pyrraline, N^{ε}-(carboxyethyl)lysine (CEL), glyoxal-lysine dimer (GOLD), methylglyoxal-lysine dimer (MOLD), and deoxyglucosone-lysine dimer (DOLD)

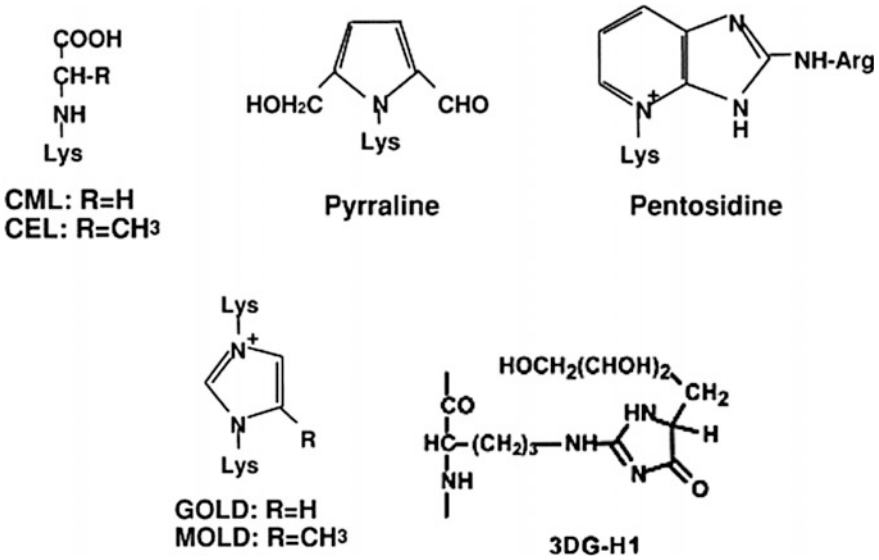

Fig. 14.3 Structure of the N^{ε}-(carboxymethyl)lysine (CML), pentosidine, pyrraline, N^{ε}-(carboxyethyl)lysine (CEL), glyoxal-lysine dimer (GOLD), methylglyoxal-lysine dimer (MOLD), and deoxyglucosone-lysine dimer (DOLD)

14.2.1.1 Impact of the Maillard Reaction

The Maillard reaction or NEB affects the colour, flavour, smell, texture, and nutritional value of foods. The interaction between proteins and reducing sugars contributes both positively and negatively to the properties, quality, and safety of foods and biological and medicinal systems. The Maillard reaction forms volatile flavour components and brown melanoidins as its high molecular weight products. These compounds are often desirable in cooking, baking, and roasting. Formation of volatile substances and brown polymers during food storage can be undesirable and lead to reduced stability, quality, and safety [e.g., formation of acrylamide, a carcinogen, during the Maillard reaction (Stadler et al. 2002)].

Maillard Reaction, Impact on Physical and Physicochemical Properties

Sugars are used as stabilizing agents for the retention of biological activity, and stabilization of protein conformation in freezing, freeze-thawing, dehydration, thermal processes, and storage. Carpenter et al. (1987) showed that the addition of 200–500 mM maltose in freeze-drying of phosphofructokinase (the sensitive enzyme in rabbit skeletal muscle) improved recovery of enzyme activity up to 80 % of the original activity and 0.9 mM ionic zinc (Zn^{2+}), with the enzyme–monosaccharide (80–100 mM glucose and 60–200 mM galactose) or–disaccharide

(30–500 mM maltose) mixtures greatly enhanced stability after freeze-drying. The results of the study indicated that the enzyme activity was not only dependent on the type of the sugar moiety present in the system, but the subunit and orientation of sugar molecules was also an important factor affecting enzyme stabilization. Carpenter and Crowe (1989) and Allison et al. (1999) demonstrated that the direct interactions of sugars (glucose and lactose) and polar groups of lysozyme protein (from egg white) via hydrogen bonding protected the protein against unfolding, which preserved the native protein structure during freeze-drying and rehydration. The Maillard reaction at a later stage (advanced Maillard reaction) caused modification of protein structure (Zhou et al. 2013), formation of non-disulfide covalently cross-linked aggregates of proteins (McPherson et al. 1988; Kato et al. 1989) and fluorescent compounds (McPherson et al. 1988; Kato et al. 1989; Yeboah et al. 1999), formation of yellow-brown colour (Lewis and Lea 1950; Kato et al. 1986; Kato et al. 1989), protein insolubility (Kato et al. 1986; Stapelfeldt et al. 1997; Yeboah et al. 1999), protein indigestibility in vitro (Öste et al. 1986), protein polymerization (Kato et al. 1989; Stapelfeldt et al. 1997; Handa and Kuroda 1999), and loss of amino acid residues (Lewis and Lea 1950; Fry and Stegink 1982; Kato et al. 1986) in food systems during storage.

Loveday et al. (2009) found that the initial and intermediate stages of the Maillard reaction (no browning formation) in milk protein concentrate-cocoa butter-glucose-glycerol-water mixtures at a component ratio of 2:1:4:1.5:1.5, as a model of a protein bar, after storage at 20 °C for 50 days, decreased the reactive lysine content, clustered protein particles, and precipitated protein, which led to hardening of the protein bars. Chen et al. (2012) showed that glucose had a higher reactivity with β-lactoglobulin for glycation than fructose in intermediate-moisture food model systems (consisting of β-lactoglobulin-glucose-glycerol-water (0.54 a_w) and β-lactoglobulin-fructose-glycerol-water (0.52 a_w) at a component ratio of 4.5:1.25:3:1.25). After storage at 35 °C for up to 49 days, their systems showed an increase in the molecular weight of the β-lactoglobulin fraction, aggregated proteins, and formation of high molecular weight protein polymers as a result of the Maillard reaction during storage. Zhou et al. (2013) studied effects of the Maillard reaction on the properties of high-protein bars [whey protein isolate (WPI)-fructose-glycerol-water (0.60 a_w) and WPI-sorbitol-glycerol-water (0.59 a_w)]. Their results showed that formation of protein aggregates by non-disulfide covalent cross-linking during storage at 25, 35, and 45 °C for 45, 14, and 3 days, respectively, occurred only in systems containing fructose (as sorbitol is a non-reducing sugar). The solubility of WPI in that system decreased after storage at 45 °C for 7 days, and formation of insoluble protein aggregates (high molecular weight polymers) via non-disulfide covalent cross-linking through the final stage of the Maillard reaction was obvious after 45 days of storage. Also, changes in texture (hardening) of the systems containing fructose correlated with the development of brown colour during storage, particularly during storage at 35 and 45 °C.

Maillard Reaction and Thermal Properties

The Maillard reaction affects thermal properties of proteins, including hydration, denaturation, and aggregation, and the glass transition of amorphous foods. Hydration refers to the interactions of protein with water (Kinsella and Whitehead 1989). Protein denaturation is alteration in the structure of a protein from its original native structure (Mulvihill and Donovan 1987). These changes in structure are restricted to changes in the secondary or higher structural level of proteins (Mulvihill and Donovan 1987). The typical thermal denaturation of proteins is unfolding of the protein structure. Unfolding of protein conformation exposes side-chain groups of amino acid residues that are buried within the native structure, which enhances hydrophobic interactions (Kinsella and Whitehead 1989). The unfolded protein molecules associate to form aggregates of denatured molecules, which can reduce protein solubility and result in coagulation and/or gelation. Aggregation and thermal gelation of protein, both of which are irreversible phenomena, result from intermolecular association of partially denatured protein chains (Mulvihill and Donovan 1987; Mulvihill and Kinsella 1987), and modifications of structure with subsequent exposure of the hydrophobic surface. The denaturation and aggregation of proteins can be induced by a number of factors such as pH, protein concentration, time and temperature of heating, and chemical reactions (e.g., Maillard reaction and lipid oxidation) (Kinsella and Whitehead 1989). Potes et al. (2013) found that products of nonenzymatic browning (dominance of advanced glycation end products) and lipid oxidation reactions in high-solids systems [WPI-olive oil-(glucose-fructose) and WPI-sunflower oil-(glucose-fructose)] increased aggregation, hydrophobicity, and oxidation (carbonyl contents) of proteins and hardening of matrixes with time. After storage for 2 weeks at 40 °C, endothermic transition of protein denaturation in these systems was absent, which showed that protein conformation was altered during storage, and denaturation transition typical of native protein was not observed on the differential scanning calorimetry (DSC) thermograms.

The addition of sugars to protein systems can stabilize proteins against thermal denaturation and increase the temperature of protein denaturation (Ball et al. 1943; Back et al. 1979; Kato et al. 1981; Boye and Alli 2000; Rich and Foegeding 2000; Potes et al. 2013), thereby postponing aggregation such that increased time and temperature are required for aggregation (Ball et al. 1943; Kato et al. 1981; Rich and Foegeding 2000; Potes et al. 2013). Back et al. (1979) showed that the presence of 28 % and 50 % (w/w) of monosaccharides (arabinose, fructose, galactose, glucose, mannose, rhamnose, and ribose), disaccharide (maltose), trisaccharides (raffinose and melezitose), and polysaccharide (dextran) in ovalbumin dispersion at pH 7 increased the peak temperature of protein denaturation measured by heating scan using DSC. The study concluded that the stabilization of protein was due to the effects of sugars on hydrophobic interactions of hydrophobic groups of the protein. Arakawa and Timasheff (1982) suggested that sugars (glucose and lactose) in aqueous solutions increased surface tension of water, promoting preferential interaction of proteins with solvent components, resulting in thermal stabilization of

the proteins. Kella and Poola (1985) found that arachin (the protein in peanuts) became more compact (had reduced viscosity) in the presence of D-xylose or D-glucose at pH 3.6. An increased hydroxyl content of the sugar (D-xylose < D-glucose) was more effective reducing thermal denaturation and aggregation of the protein. Rich and Foegeding (2000) found that 500 mM ribose or 500 mM lactose increased the peak temperature of protein denaturation [14 % (w/w) whey protein isolate dispersion] and that both sugars showed an ability to inhibit the heat-induced aggregation of proteins. Edelman et al. (2015) recently proposed a "templating" concept to explain the effect of sugar stereochemistry on water structure and protein thermal stability. They showed that sugar isomers that better fit the hexagonal structure of ice served as better templates for cooperative hydration in solution, and thus had higher hydration numbers and provided better protection against denaturation of globular proteins.

The extent of interactions between proteins and sugars through the Maillard reaction affects thermal properties of the proteins in dry systems. Kato et al. (1981) found that freeze-dried ovalbumin-glucose at 65 % RH and at 50 °C for up to 6 days of storage, increased denaturation and aggregation temperatures of the protein. Kato et al. (1981) found increased solubility of the protein at the initial stage of the reaction but decreased soluble protein content at the final stages of the reaction, and increased formation of aggregated proteins through the Maillard reaction. Their results also showed that after storage with glucose for 6 days, ovalbumin became more heat-sensitive (having lower temperature and heat enthalpy of protein denaturation) than the systems with and without glucose before storage and the system without glucose after storage for 6 days. The formation of protein–glucose complexes and cross-linking as a result of the Maillard reaction promoted the unfolding and insolubilisation of the proteins, respectively. Easa et al. (1996) showed that the production of acidic products from Maillard reaction during heating at 90 °C for up to 75 min, decreased the pH of bovine serum albumin–ribose or–xylose systems, which influenced the thermal gelation or aggregation of the protein. Decreasing of protein solubility and formation of high molecular weight protein polymers (36–45 kDa) in high-solids systems was also reported by Potes et al. (2013, 2014), and was explained by intermolecular hydrophobic interactions of protein–lipid–sugars components during storage at 40 °C for up to 14 weeks. Their results indicated protein modification, polymerization, and component interactions via glycation (nonenzymatic glycosylation or Maillard reaction) leading to non-disulfide covalent cross-linking and insolubilisation of proteins during storage.

The Maillard reaction affects glass transition of amorphous foods. Systems containing amorphous solids were related to physical instability (stickiness, collapse, crystallization, and oxidation) of the materials. Roos et al. (1996a) found that the Maillard reaction, a temperature-dependent exothermal phenomenon, in freeze-dried amorphous skim milk [4.9 % (w/w) lactose and 3.4 % (w/w) protein] and lactose-hydrolyzed skim milk [2.45 % (w/w) glucose, 2.45 % (w/w) galactose, 3.4 % (w/w) protein] produced water that plasticized the amorphous material, thereby decreasing the glass transition temperature (T_g). These results also showed

that the glass transition of the systems became more broadened with increasing isothermal (at 100, 110, 120, 130, and 140 °C) holding time in the DSC because of the formation of high molecular weight polymeric compounds. The formation of polymeric compounds was likely to increase T_g of the material. Increased water content and decreased T_g of the materials, as the result of the Maillard reaction, at each temperature were linear against heating time.

Maillard Reaction and Protein Structure

The effects of the Maillard reaction on the chemical properties of proteins have been related to sulfhydryl group content and to conformational and structural changes in proteins. Watanabe et al. (1980) showed that the Maillard reaction promoted unfolding of ovalbumin and reduced α-helix conformation in the secondary structure of the proteins. The system contained lysine and arginine residues in freeze-dried ovalbumin with glucose (65 % RH) during storage at 50 °C for 18 days. Kato et al. (1987) showed that 3-deoxyglucosone (dicarbonyl compound from the Maillard reaction between butylamine and glucose) induced polymerization of proteins by intermolecular cross-linking of lysine, arginine, and tryptophan residues in freeze-dried mixtures of lysozyme or acetylated lysozyme with glucose during storage for 3 days at 50 °C and 75 % RH. The 3-deoxyglucosone was a cross-linker for protein polymerization through the Maillard reaction. Handa and Kuroda (1999) found that spray-dried egg white (6.5 % water content) with glucose (35 % RH) after storage at 55 °C for up to 12 days showed the formation of protein-glucose complexes in the initial stages of the Maillard reaction (i.e., a decrease in glucose and available lysine content); an increase in browning, sulfhydryl groups content, polymerization and aggregation of proteins at the later stage of the reaction; and a decrease in pH and α-helix content with increasing thermal storage time. Their results showed that the proteins in egg albumin were unfolded through the Maillard reaction. The polymerization or aggregation of proteins via the Maillard reaction involved the covalent rather than the disulfide bonds.

14.2.1.2 Control and Inhibition of the Maillard Reaction

The Maillard reaction is dependent on a_w and water content (Labuza and Dugan 1971), and it is affected by numerous factors including the chemical structure of sugars and amino acids (Lewis and Lea 1950; Pomeranz et al. 1962; Ashoor and Zent 1984; Kato et al. 1986; Carpenter et al. 1987), concentration or quantitative ratio of amino groups to reducing sugar (Kato et al. 1986), pH (Pomeranz et al. 1962; Ashoor and Zent 1984; Morita and Kashimura 1991; Baxter 1995), storage time (Kato et al. 1986), temperature (Fry and Stegink 1982; Baxter 1995), oxygen (Fu et al. 1992; Hayase et al. 1996), porosity and collapsed structure of the matrix (Karmas et al. 1992; Buera and Karel 1995; Schebor et al. 1999; Burin et al. 2004),

and crystallization of sugar in the systems (Karmas et al. 1992; Burin et al. 2004; Buera et al. 2005; Drusch et al. 2006).

Lewis and Lea (1950) showed that aldopentoses (xylose and arabinose) reacted with casein at 25 °C over a 16-day period more rapidly than aldohexose (glucose), followed by aldodisaccharides (lactose and maltose) and ketohexose (fructose). The development of brown colour in the casein–sugar mixtures at 37 °C followed the order xylose > arabinose > glucose > lactose, maltose, and fructose, which showed that pentoses caused more rapid damage to the amino groups of casein proteins than aldohexose and ketohexose. Bunn and Higgins (1981) showed that the reactivity of sugars with proteins in glycation was more dependent on the open (carbonyl) structure than on the ring (hemiacetal or hemiketal) structure due to the high stability of the ring structure, and aldoses showed higher reactivity than ketoses. Pilková et al. (1990) showed that the rate of browning of Heyns products was slower than that of Amadori products. Morita and Kashimura (1991) suggested that phosphorylated monosaccharides produced higher levels of browning and fluorescence than their monosaccharide units. Baxter (1995) reported results in agreement with Lewis and Lea (1950) that an aldohexose (glucose) was more reactive in interaction with amino acids and formed browning at pH 7.5 after heating at 128 °C for 225 s, compared to a ketohexose (fructose) and aldodisaccharides (lactose and maltose). Kwak and Lim (2004) showed that the intensities of browning from interactions between sugars (maltose, fructose, glucose, arabinose, and xylose) and amino acids (aspartic acid, glutamic acid, alanine, leucine, isoleucine, valine, proline, serine, cysteine, phenylalanine, arginine, and lysine) was in the order xylose > arabinose > glucose > maltose > fructose, and that lysine had the highest reactivity with sugars, followed by arginine and cysteine. The sulphur-containing amino acids and peptides such as cysteine and glutathione were effective in preventing long-term food browning (both enzymatic and nonenzymatic browning reactions) under typical food storage and processing conditions (Molnar-Perl and Friedman 1990).

Ashoor and Zent (1984) found that the maximum pH value for formation of brown colour in 0.005 M L-amino acids (lysine, alanine, and arginine) and 0.005 M glucose or fructose or α-lactose at 1:1 ratio in 8 mL of 0.05 M carbonate buffer solutions (pH 8 to 12) after heating at 121 °C for 10 min was pH 10.0. They classified common amino acids and amides into three groups according to formation of brown colour during heating with reducing sugars (D-glucose, D-fructose, D-fructose, D-ribose, and α-lactose). The first group showed strong colour and included L-amino acids lysine, glycine, tryptophan, and tyrosine. The second group showed intermediate browning and included L-amino acids proline, leucine, isoleucine, alanine, hydroxyproline, phenylalanine, methionine, valine, and the amides L-glutamine and L-asparagine. The third group was the least effective in browning and included L-amino acids histidine, threonine, aspartic acid, arginine, glutamic acid, and cysteine. However, the conclusions of these studies were based on results of experiments conducted using buffer solutions and the results from experiments performed under dry heating or in solid systems may vary. Bell (1997) showed that using different types (citrate and phosphate buffers) and concentrations (0.02 up to

0.5 M) of buffer to maintain the pH at 7.0 in glucose-glycine (at 1:1 ratio) systems affected the initial degradation of amino acids and formation of brown pigment in the Maillard reaction. These results showed that the glucose-glycine showed a higher rate of glycine loss and formation of brown pigment in the phosphate buffer than in the citrate buffer, especially at high reactant concentrations. Yeboah et al. (1999) found that the initial rate of utilization of amino groups of bovine serum albumin was higher by D-glucose in glycation than by D-fructose under dry heating conditions (heated freeze-dried protein–sugar mixture (0.65 a_w) at 50 °C). The study was carried out in the presence of oxygen or nitrogen, and showed that the reaction of sugars and amino groups was slower in the presence of nitrogen than oxygen at the initial stage of the Maillard reaction, evidencing the role of the oxidation reaction in certain Maillard pathways. D-fructose was more sensitive to the presence or absence of oxygen than D-glucose.

Burin et al. (2004) showed that the brown colour development in the Maillard reaction between whey protein and lactose was higher in a compressed structure of the system than in a porous structure, which was attributed to the diffusion of water formed in the browning reaction. Their results showed that crystallization of lactose also accelerated browning, and thus the use of a lactose–maltodextrin (MD) mix in the systems retarded lactose crystallization (the reduced molecular mobility of lactose) and also the brown colour development.

Nonenzymatic browning reaction can be inhibited and controlled by several means, e.g., using the following principles:

(i) The addition of reagents that can combine with or eliminate carbonyl groups such as aminoguanidine (prevented formation of fluorophores in the reaction), antioxidants [phenolic compounds, butylated hydroxytoluene (BHT)], sodium bisulfite, metal chelating agents [e.g., diethylenetriamine pentaacetic acid (DETAPAC), citrate, deferoxamine mesylate, and superoxide dismutase; inhibited the browning and the formation of fluorophores], sodium cyanoborohydride ($NaBH_3CN$; decreased Schiff bases, inhibited Amadori rearrangement and formation of fluorescence), pyridoxamine (one form of vitamin B_6; inhibited post-Amadori steps, blocking oxidative degradation of Amadori intermediate, and scavenged reactive carbonyl compounds from the degradation of sugars, lipids, and amino acids) (Molnar-Perl and Friedman 1990; Pilková et al. 1990; Morita and Kashimura 1991; Voziyan and Hudson 2005; Uribarri et al. 2010).

(ii) Lowering of a_w or water content and temperature during processing and storage of food products (Karmas et al. 1992; Schebor et al. 1999; Burin et al. 2004).

(iii) Lowering of pH to acidic conditions below pH 7 (Pomeranz et al. 1962; Fox et al. 1983; Molnar-Perl and Friedman 1990; Pilková et al. 1990; Ajandouz and Puigserver 1999; Kwak and Lim 2004; Uribarri et al. 2010).

(iv) Removal or conversion of one of the reactants of the Maillard reaction, such as replacement of reducing sugars with nonreducing ingredients (sucrose,

trehalose, sugar alcohols such as glycerol, sorbitol, and maltitol) (Liu et al. 2009a; Zhou et al. 2013).

(v) Removal of metal ions such as Zn^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+} and Cu^{2+} that may accelerate formation of Maillard reaction products, such as chromophores and fluorophores, by the oxidative pathway and development of browning (Fry and Stegink 1982; Pilkova et al. 1990; Morita and Kashimura 1991; Makris and Rossiter 2000; Kwak and Lim 2004).

(vi) Addition of mono- and divalent salt ions such as Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+}, which can delay and inhibit the development of browning and prevent formation of acrylamide (Kwak and Lim 2004; Gökmen and Şenyuva 2007).

(vii) Removal or replacement of oxygen with an inert gas to reduce the rate of oxidation reactions that promote the Maillard reaction, thereby decreasing the formation of the browning products (Ahmed et al. 1986; Fu et al. 1992).

(viii) Reduction of structural changes (maintenance physical stability) and deteriorative reactions that can accelerate the Maillard reaction such as collapse or shrinkage (Karmas et al. 1992; Buera and Karel 1995; Schebor et al. 1999), sugar crystallization (Karmas et al. 1992; Burin et al. 2004; Buera et al. 2005; Drusch et al. 2006), state of the matrix (Lievonen et al. 1998), phase separation (Lievonen et al. 1998), and lipid oxidation (Hidalgo and Zamora 2000; Zamora and Hidalgo 2005).

14.2.2 Lipid Oxidation

Lipid oxidation is a deteriorative reaction that often occurs during food processing and storage, and ageing in vivo. The breakdown or decomposition reactions of unsaturated fatty acids leads to the development of undesirable off flavours (rancidity), formation of toxic compounds, and loss of flavour, nutritional value, colour, and physical stability of foods (Greene 1969; Labuza and Dugan 1971). Lipid oxidation shows its highest rates between a_w of 0.60 and 0.80. That a_w range agrees with the highest rates reported for the nonenzymatic browning reaction, but oxidation reactions also show high rates at low a_w ($a_w < 0.1$) (Labuza and Dugan 1971) (Fig. 14.4). Lipid oxidation reactions can be divided into three stages: initiation, propagation, and termination, following the free radical mechanism shown in Fig. 14.5. The initiation stage entails the formation of a free radical (an unpaired-electron compound). Formation of the free radicals is initiated by catalysts such as light, oxygen, and water (Labuza et al. 1969). The propagation stage is a chain of reactions of free radicals with oxygen or unsaturated fatty acids. Propagation transfers and transforms the free radicals through a chain reaction or an autocatalytic process that produces compounds causing rancidity [volatile aldehydes and ketones (hydroperoxides)]. The termination stage includes reactions between free radicals to form non-radical end products. The general pathways of

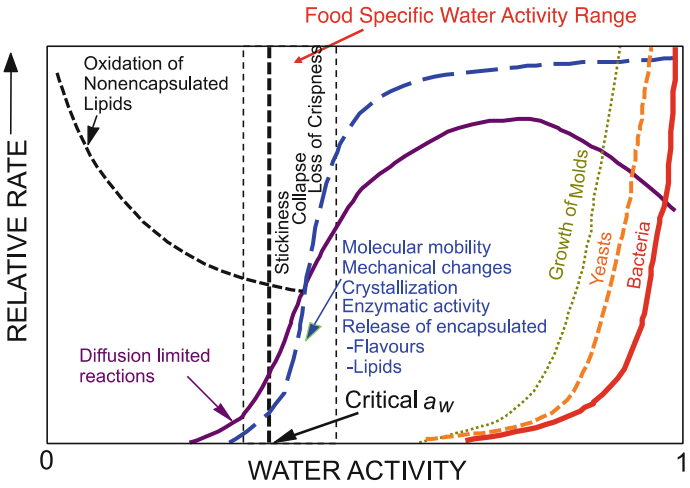

Fig. 14.4 Stability map of foods showing relative rates of physical changes (related to molecular mobility, crystallization, and deteriorative changes) and chemical reactions against a_w (adapted from Roos et al. 1996b)

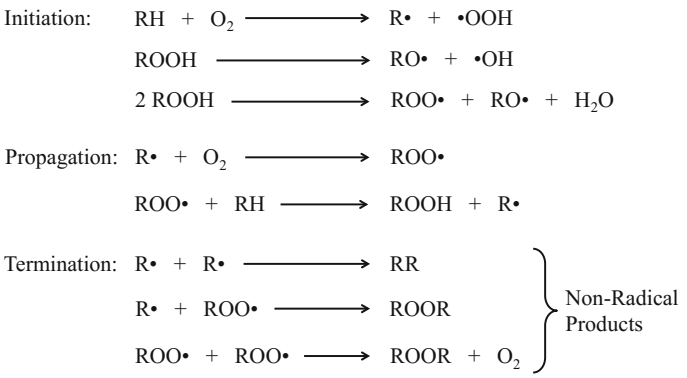

Fig. 14.5 The free radical mechanism in lipid oxidation as reported by Labuza and Dugan (1971) and Karel (1973). In this diagram, RH = unsaturated fatty acid or substrate, R· = alkyl radical (substrate), ROOH = hydroperoxide (e.g., hexanal, pentanal, and malonaldehyde), RO· = alkoxyl radical (breakdown product of lipid hydroperoxides), ·OOH = hydroperoxyl radical, ·OH = hydroxyl radical, ROO· = peroxyl radical, and RR and ROOR = stable end product with no free radical

lipid oxidation and the extent of the reaction against time are shown in Figs. 14.6 and 14.7, respectively.

The consumption of lipid oxidation products in a diet, especially products from lipid peroxidation (e.g., malondialdehyde and 4-hydroxynonenal), increases the risks of apoptosis or damage to phospholipid (lipoprotein) membranes (Karel 1973;

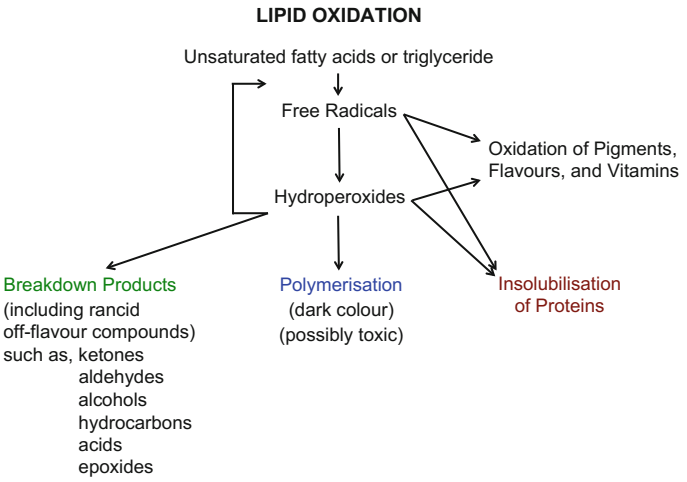

Fig. 14.6 The general pathways of lipid oxidation (Labuza and Dugan 1971) [Kinetics of lipid oxidation in foods, Labuza and Dugan (1971), reprinted by permission of the Taylor & Francis Ltd.]

Spiteller 2005), and to numerous diseases, such as Alzheimer's disease (neuron degeneration in brain) (Sayre et al. 1997; Markesbery and Lovell 1998), atherosclerosis and coronary artery diseases (Addis 1986; Stringer et al. 1989; Plachta et al. 1992), liver diseases (Yagi 1987; Rouach et al. 1997), formation of ageing pigments or fluorescent complexes (Karel 1973), rheumatoid arthritis (Baskol et al. 2006), and skin inflammation and acne (Briganti and Picardo 2003). The lipid peroxidation products (including free radicals) can attack the endothelial cells of blood vessels, intact organs and tissues (dysfunction of the membrane), increasing platelets aggregation, and accumulate in the blood (attack the blood vessels) in vivo (Yagi 1987). The malondialdehyde (dicarbonyl compound) and 4-hydroxynonenal (polar lipid) can react with amino acids (e.g., arginine, lysine, cysteine, histidine, methionine, and tyrosine) in proteins via either intra- or intermolecular cross-linking, leading to chemical modification of proteins (oxidative protein damage, increased protein carbonyl content), polymerization, browning, and formation of fluorescent complexes (Karel 1973; Requena et al. 1996; Rouach et al. 1997) in ageing and neurodegenerative and chronic diseases. The mechanisms of free-radicals transfer to the proteins and amino acids via lipid peroxidation products are given below (Schaich and Karel 1976):

$$\mathrm{ROOH + PH \longrightarrow ROOH\text{----}HP} \begin{cases} \nearrow \mathrm{RO\bullet + P\bullet + H_2O} \\ \searrow \mathrm{RO\bullet + \bullet OH + PH} \end{cases}$$

$$\mathrm{PH + RO\bullet \longrightarrow P\bullet + ROH}$$

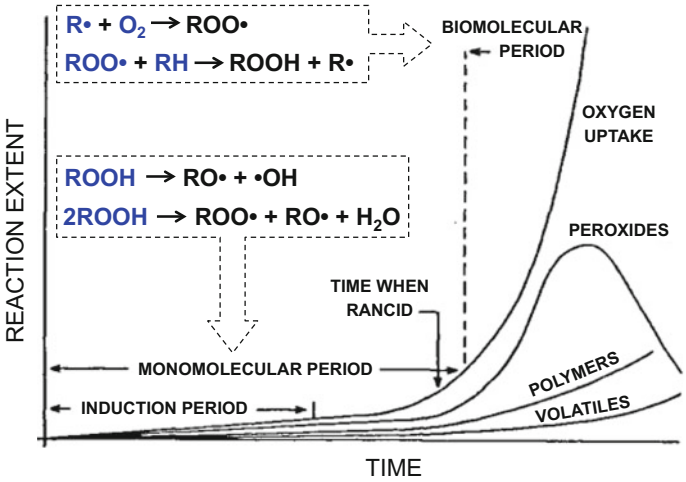

Fig. 14.7 Extent of lipid oxidation reactions as a function of time, adapted from Labuza and Dugan (1971) reprinted by permission of the Taylor & Francis Ltd

where
ROOH = lipid hydroperoxide
PH = nitrogen or sulphur centres of reactive amino acid residues of the protein
RO· = alkoxyl radical (breakdown product of lipid hydroperoxides)
P· = protein radical
·OH = hydroxyl radical
ROH = lipid hydroxide

It should be noted, however, that lipid peroxidation reactions could produce carbonyl compounds (e.g., glyoxal, CML, and GOLD), as shown in Fig. 14.2. Such carbonyl compounds are typical products of the Maillard reaction in food and biological systems (Requena et al. 1996). Indeed, oxidation and the Maillard reaction can be interrelated and concomitant.

14.2.2.1 Impact of Lipid Oxidation

The effects of lipid oxidation products on properties of high-solid foods have been studied by several authors. Labuza et al. (1969) and Zirlin and Karel (1969) investigated effects of oxidized lipids on proteins during storage at various relative humidities. Their studies revealed that aggregation [decreased protein solubility in water and acetate buffer (pH 4.8); increased protein hydrophobicity] and oxidation of the protein in a freeze-dried gelatine-methyl linoleate system increased with increasing RH (up to 60 % RH) during 6 days of storage at 50 °C, although less

lipid oxidation occurred at high relative humidities. The system stored at a low RH (maximum for lipid oxidation) showed a lower melting temperature for a gel prepared using the system. That is, the degraded protein had lower molecular weight fragments (protein scission), which gave an increased solubility of the gelatine in ethanol–0.8 M NaCl mixtures. Zirlin and Karel (1969) concluded that at the high relative humidities, cross-linking of protein radicals [from interaction of lipid peroxyl radical (ROO·) with protein (PH); ROO· + PH → ROOH + P·] was favoured and predominated over the oxidative scission of the protein. Conversely, in the dry state (low RH) protein radicals interacted with oxygen to form protein peroxyl radicals (P· + O_2 → POO·), and subsequent scission of the –N–C– bonds.

The reaction of lipid peroxidation products (free radicals, lipid hydroperoxides, and aldehyde derivatives) and proteins in dehydrated food systems may result in losses of amino acids (cysteine, histidine, lysine, methionine, tryptophan, tyrosine) leading to damaged proteins (Roubal and Tappel 1966a; Zirlin and Karel 1969; Roubal 1971; Nielsen et al. 1985; Refsgaard et al. 2000; Wu et al. 2010), browning (Zirlin and Karel 1969; Potes et al. 2014), copolymerization of peroxidized lipids and proteins (lipid–protein complexes) (Roubal and Tappel 1966b; Kanner and Karel 1976; Funes et al. 1982), formation of free protein radicals (Roubal and Tappel 1966a, b; Zirlin and Karel 1969; Karel et al. 1975), formation of disulfide bonds (Hidalgo and Kinsella 1989), protein oxidation [formation of protein-carbonyl derivatives (Stadtman 1992; Dalle-Donne et al. 2003; Potes et al. 2013)] (Labuza et al. 1969; Zirlin and Karel 1969; Refsgaard et al. 2000), denaturation and aggregation [at high a_w (0.75 a_w) leading to decreasing of protein solubility] (Zirlin and Karel 1969; Karel 1973; Kanner and Karel 1976; Leake and Karel 1982; Potes et al. 2013; 2014), and scission of proteins leading to increased protein solubility and decreased viscosity of protein dispersions (Roubal and Tappel 1966b; Labuza et al. 1969; Zirlin and Karel 1969) as well as production of covalently cross-linked protein–protein polymers (Roubal and Tappel 1966b; Karel 1973; Kanner and Karel 1976; Leake and Karel 1982; Potes et al. 2014). The polymerization, cross-linking, and scission reactions of proteins depend on the a_w of the system. Zirlin and Karel (1969) showed that the protein scission evidenced by decreased molecular weight and increased content of protein amide groups occurred when proteins were exposed to peroxidized lipids, but the reaction was inhibited after increasing the a_w of the system. Kanner and Karel (1976) showed that a high a_w (0.75 a_w) promoted cross-linking and aggregation or insolubilisation of proteins in a system containing lipid peroxides and proteins. Jones et al. (2005) found that the presence of silicone oil in a protein dispersion caused conformational changes, alterations in thermal stability, and acceleration of protein [bovine serum albumin (BSA) and ribonuclease A] aggregation at pH 4.5, 6.5, and 7.2 before and after storage at 45 °C.

The polymerization, cross-linking, and formation of lipid–protein complexes from the oxidation reaction also alter water sorption properties, physical structure and physicochemical properties of dried foods (Koch 1962; Karel 1973). The higher molecular weight materials exhibit higher sorbed water content at low a_w than the lower molecular weight components (Roos 1993; Potes et al. 2012).

Therefore, the oxidation of lipid and protein resulting in polymerization, protein aggregation, protein–protein cross-linking, formation of polymeric components has potential in lowering the a_w of systems. Changes in protein conformation favouring hydrophobic interactions with oil and subsequent increase in hydrogen bonding of water to exposed hydrogen bonding sites can be another explanation for lowering of the a_w values. Degradation of lipids (lipolysis) by lipases or in the presence of radicals, and the thermal treatment could also release fatty acids from triglycerides (consumption of water in the systems). Highly oxidized unsaturated fatty acids and impurities of components used in the system can lead to a brown coloured surface and hardening of the protein and the matrix.

Karel (1973) reported that lipid–protein complexes in food systems affected stiffness (rigor mortis) in the ageing of meat, and loss of protein solubility and browning during storage of frozen fish and dried muscle foods. The interactions of radicals produced by lipid oxidation with proteins are shown in Fig. 14.8. Wu et al. (2010) found that the structure and properties of a soy protein isolate were modified by acrolein (aldehyde product of lipid peroxidation) in a sodium phosphate buffer (pH 7.4) dispersion during incubation at 25 °C in the dark for 24 h. Acrolein caused denaturation (unfolding and exposed hydrophobic residues) and aggregation (hydrophobic interactions) of the protein, decreased protein solubility in water (pH 7), increased protein carbonyls, decreased total sulfhydryl (SH) content (formation of disulfide bonds and sulphur-containing oxidation products), formation of covalently cross-linked proteins (with no disulfide bonds), loss of α-helix structure, and increased β-sheet structure. Cucu et al. (2011) showed that incubation of solutions of whey protein isolate with oils (fish, sunflower, soybean, and olive oil) at 70 °C for up to 48 h resulted in an increase of protein bound carbonyls [amino acids react with lipid hydroperoxides (e.g., 4-hydroxy-2-nonenal and malondialdehyde) or with reactive carbonyl derivatives (e.g., ketoamines, ketoaldehydes, deoxyosones) generated from the Maillard reaction (Berlett and Stadtman 1997; Refsgaard et al. 2000)]. The carbonyl content was dependent on the oil used; the carbonyl content decreased in the order fish oil > soybean oil > sunflower and soybean oil > olive oil. The formation of high molecular weight aggregates of the protein and covalent cross-linking of the protein, as well as increased lipid oxidation, increased with storage time. Potes et al. (2013) showed that increasing the level of unsaturation of oil in high-solids formulations increased oxidation of proteins (increased carbonyls content) in whey protein isolate (WPI) during storage, especially at high temperatures, and they found that mixing oil [olive oil (OO) or sunflower oil (SO)] with proteins (0.35 or 0.34 a_w, respectively)] increased the onset temperature of the endothermic transition of protein denaturation from 55 °C [10 % (w/w) protein dispersion of WPI alone] to 61 °C [10 % (w/w) protein dispersion of WPI-OO or–SO]. The presence of OO or SO in the dry mixes induced protein aggregation (increased heat of protein aggregation) before storage at 20 and 40 °C. Increasing lipid oxidation increased protein aggregation (decreased heat of protein aggregation), as was shown by the reduced size of exothermic protein aggregation transitions during storage. Proteins became more heat-sensitive, as observed by lowering

of the onset temperature of protein denaturation, and lowering of the heat of protein denaturation after storage for 14 weeks at both 20 and 40 °C. Potes et al. (2014) found colour changes, decreased a_w, formation of protein aggregates, and covalent cross-linking in the WPI-oil (OO or SO) systems after storage at 40 °C.

14.2.2.2 Occurrence and Inhibition of Lipid Oxidation

The rate of lipid oxidation in high-solids systems depends on the amount of unsaturated fatty acid moieties or double bonds in the lipid molecules (as opposed to the total fat content) (Labuza and Dugan 1971), a_w or water content (Labuza et al. 1969, 1972; Labuza and Dugan 1971), enzymes (Rhee et al. 1987), food components (Labuza et al. 1969; Sakanaka et al. 2004; Peña-Ramos and Xiong 2003), glass transition temperature (Shimada et al. 1991), oxygen (Labuza and Dugan 1971; Ordonez and Ledward 1977), pH (Richards and Hultin 2000), processing and storage temperature (Labuza and Dugan 1971; Morcira et al. 1997; Krokida et al. 2000; Jakobsen and Bertelsen 2000), physical structure (e.g., porosity, size, and surface area) of the material (Labuza et al. 1969; Pinthus et al.

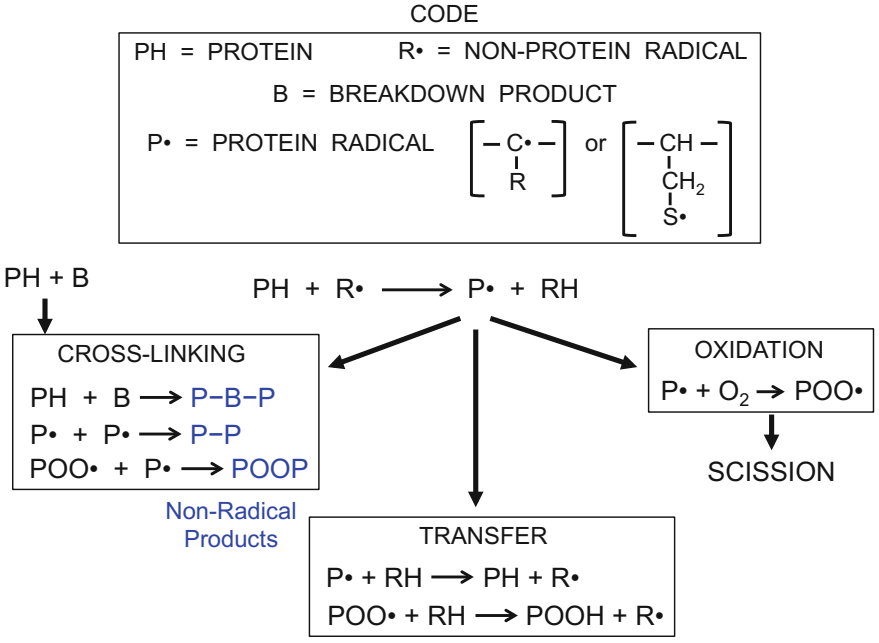

Fig. 14.8 Schematic representation of reactions of proteins with peroxidising lipids (R· and B) (Karel et al. 1975) [Reprinted with permission from Karel et al. (1975), Copyright 1975 American Chemical Society]

1995; Morcira et al. 1997; Krokida et al. 2000), crystallization of components (Shimada et al. 1991; Drusch et al. 2006), and Maillard reaction (Kirigaya et al. 1968; Bucala et al. 1993; Yen and Hsieh 1995; Mastrocola and Munari 2000; Breitling-Utzmann et al. 2001).

Labuza et al. (1969) demonstrated that gelatine-methyl linoleate systems (60 % RH) at 50 °C showed enhanced oxidation of protein (aggregation and reduced solubility of protein), although the lipid oxidation was reduced. They reported that the protein reacted with the peroxides through a free radical mechanism, which reduced the lipid oxidation reaction rate. Labuza and Dugan (1971) found both positive and negative effects of water on the rate of lipid oxidation. Water as a solvent mobilized hydrophilic reactants. Water could interact with metal catalysts making them less effective, as well as forming hydrogen bonds with hydroperoxides with a consequent reduction of the rate of lipid oxidation at the initiation stage. The solvent and mobilization properties of water are pronounced at high water activities (0.55 to 0.85 a_w) as a result of mobilization of catalysts (increasing rate of lipid oxidation).

Foods containing proteins {e.g., egg proteins (Sakanaka et al. 2004), gelatine (Labuza et al. 1969), dairy and other proteins [e.g., bovine serum albumin (BSA) (Gebicki and Gebicki 1993; Refsgaard et al. 2000), lactoferrin (Gutteridge et al. 1981; Shinmoto et al. 1992), caseins (Cervato et al. 1999; Hu et al. 2003), soy protein isolates (Hu et al. 2003), and whey proteins (Colbert and Decker 1991; Hu et al. 2003)], and protein hydrolysates (Shahidi and Amarowicz 1996; Suetsuna et al. 2000; Peña-Ramos and Xiong 2003; Sakanaka et al. 2004)} can retard or inhibit lipid oxidation, because of the chelating properties for transition metal ions (iron-binding capacity) and free radical scavenging by amino acids and peptides (Karel et al. 1975; Gutteridge et al. 1981; Shinmoto et al. 1992; Gebicki and Gebicki 1993). Systems containing carbohydrates, sugars, and caramelization products can also retard lipid oxidation (Sims et al. 1979; Benjakul et al. 2005; Drusch et al. 2006). In an aqueous emulsion system, the use of a sugar (sucrose, dextrose monohydrate, sorbitol, and glycerol) at a high concentration (up to 67 %) decreased the concentration of oxygen in the aqueous phase, and decreased the diffusion of oxygen in the system [because of the increased viscosity of the aqueous phase (physical inhibition)] (Sims et al. 1979). In an amorphous system, retarded lipid oxidation was attributed to the unique binding properties of sugars to dienes (Drusch et al. 2006). The caramelization products from heated sugars (D-ribose, D-fructose, D-glucose, and D-xylose) acted as antioxidants (free radical scavenging and chelating activities). Such compounds effectively retard and inhibit lipid oxidation in food systems (Benjakul et al. 2005).

Products from the Maillard reaction can either promote or reduce lipid oxidation. Amadori products were shown to react with phospholipids (biological membranes), leading to increased lipid oxidation in vivo (Bucala et al. 1993; Breitling-Utzmann et al. 2001). Several authors reported that formation of brown pigments in the Maillard reaction reduced lipid oxidation (Kirigaya et al. 1968; Yen and Hsieh 1995; Mastrocola and Munari 2000). Such inhibition of lipid oxidation was dependent upon brown colour intensity (Kirigaya et al. 1968; Yen and Hsieh 1995).

Water-soluble products (from a condensation step at the early stage), hydroxymethylfurfural (intermediate product), and melanoidins (brown polymers at the final stage) of the Maillard reaction can act as free radical scavengers and as reducing (donation of hydrogen atoms) and chelating agents (Gomyo and Horikoshi 1976; Kirigaya et al. 1968; Ames 2001; Yilmaz and Toledo 2005; Chen et al. 2009). Conversely, the Maillard reaction can be promoted by the presence of oil and its oxidation products (Mastrocola et al. 2000).

Lipid oxidation can be controlled and prevented in the following ways:

(i) Addition of antioxidants for scavenging free radicals or as reducing agents, e.g., butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tertiary-butylated hydroquinone (TBHQ), propyl gallate (PG), lecithin, phenolic compounds [ascorbic acid (vitamin C), α-tocopherol (vitamin E), xanthones, carotenoids (β-carotene, lutein), gallic acid, flavonoids (e.g., anthocyanins, quercetin, and catechins)] (Labuza et al. 1969; Sherwin 1978; Rice-Evans et al. 1997; Balasundram et al. 2006).

(ii) Controlling factors that increase mass transfer of oil (e.g., water loss, oil uptake) into the products during processing, such as low initial water content, low viscosity of oil, physical structure of products (low porosity or low pore size distribution, low surface area or small size, and high thickness of materials), high heating temperature, and short heating time (Pinthus et al. 1995; Morcira et al. 1997; Krokida et al. 2000).

(iii) Controlling a_w or water content, temperature, and storage time (Labuza et al. 1969; Labuza and Dugan 1971; Nielsen et al. 1985; Sun et al. 2002).

(iv) Lowering the pH to acidic conditions (Decker and Welch 1990; Richards and Hultin 2000).

(v) Removal of transition metals and minerals, especially the predominant pro-oxidant materials such as cobalt, copper, iron, magnesium, manganese (Labuza et al. 1969; Sherwin 1978).

(vi) Removal of enzymes (e.g., lipases and lipoxygenases) in animal and plant tissues. Enzymes strongly catalyze oxidative decomposition of fats and oils (Sherwin 1978).

(vii) Removal or replacement of oxygen with an inert gas (nitrogen) to reduce the rate of oxidation reactions in packages (Greene 1969; Labuza and Dugan 1971; Nielsen et al. 1985).

(viii) Minimizing light and UV exposure during storage (Wishner 1964; Sattar et al. 1975).

(ix) The use of fats and oils that contain low levels of unsaturated fatty acids.

(x) Reduction of unsaturated fatty acids by hydrogenation (addition of pairs of hydrogen atoms to the double bonds) to prevent flavour deterioration and to improve oxidative stability (increased oil melting temperature), although this reaction converts liquid oils to semisolid fats (Coenen 1976; Emken 1984), and incomplete reduction leads to formation of trans fatty acids, which have been associated with health problems (Remig et al. 2010).

(xi) Reduction of structural changes and maintenance physical stability as a liquid-like structure with high molecular mobility enhances lipid oxidation (Labuza et al. 1969).

14.2.3 Protein Oxidation

Protein oxidation results in covalent modification of the protein (irreversible modification) structure. Protein oxidation is induced either directly by reactive oxygen species, especially free radicals of oxygen [i.e., superoxide ($O_2^{\cdot -}$), hydroxyl radical (·OH), hydroperoxyl radical (HOO·), alkoxyl radical (RO·), peroxyl radical (ROO·), nitric oxide (NO·), sulfinyl radical (RSO·), and thioperoxyl radical (RSOO·). (Stadtman and Berlett 1998)] or indirectly by reactions with secondary products of oxidation (Rivett et al. 1985; Starke-Reed and Oliver 1989; Stadtman 1992; Shacter 2000). The reactive oxygen species are generated in glycation or glycoxidation or the Maillard reaction, irradiation (γ-rays, X-rays, and UV), lipid peroxidation and free radical breakdown products (e.g., hydroxyl, alkoxyl, and peroxyl radicals), and inflammatory reactions (neutrophil macrophages) (Stadtman and Levine 2000). The lipid peroxidation products including free radicals, lipid hydroperoxides, and reactive aldehyde derivatives [e.g., 4-hydroxy-2-alkenals (4-hydroxynonenal and 4-hydroxyhexenal), dicarbonyls (glyoxal, methylglyoxal, and malondialdehyde), unsaturated aldehydes (acrolein), and saturated aldehydes (ethanal, propanal, and hexanal) (Fig. 14.9)] are most likely responsible for the damage to protein and biological membranes (Chio and Tappel 1969; Uchida and Stadtman 1992; Stadtman and Berlett 1998). Glyoxal and methylglyoxal are also produced in the Maillard reaction as shown in Fig. 14.2.

Lipid peroxidation products and an increase in protein oxidation can inactivate sulfhydryl groups and proteases (e.g., ribonuclease A, brain, and liver enzymes) or inhibit the specific (binding) activity of enzymes (Chio and Tappel 1969; Rivett et al. 1985; Starke-Reed and Oliver 1989; Carney et al. 1991). Such enzymes are responsible for the degradation of the oxidized (modified or denatured) forms of proteins (Chio and Tappel 1969; Starke-Reed and Oliver 1989; Stadtman et al. 1992). Protease (enzyme) activities are not responsible for the increase of oxidized proteins during ageing (Starke-Reed and Oliver 1989). A number of amino acid residues of proteins, such as arginine, cysteine, histidine, leucine, lysine, phenylalanine, methionine, proline, threonine, tryptophan, and tyrosine, are highly sensitive to attacks by reactive oxygen in free radical species and lipid peroxidation products (Creeth et al. 1983; Uchida and Stadtman 1992; Stadtman and Berlett 1998; Stadtman and Levine 2003; Lund et al. 2008). The oxygen free radical-mediated oxidation of methionine forms methionine sulfoxide and methionine sulfone, and cysteine forms cysteine disulfides and sulfenic acid (Garrison 1987).

Oxidation of enzymes and some amino acids (lysine, arginine, threonine, and proline) leads to the formation of carbonyl (aldehydes and ketones) derivatives

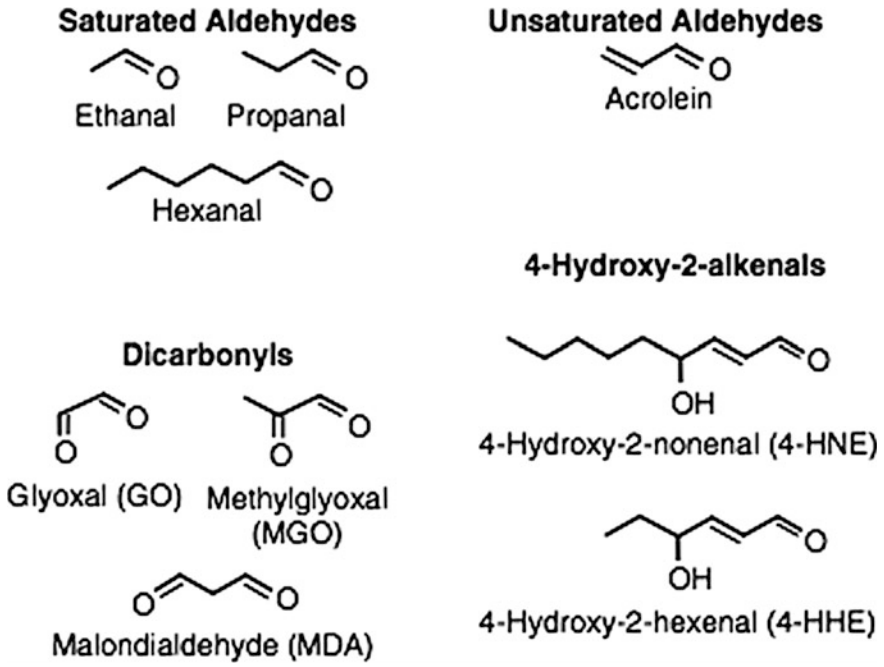

Fig. 14.9 Structure of some aldehydic compounds derived from lipid peroxidation (Negre-Salvayre et al. 2008) (Reprinted with permission from Negre-Salvayre et al. (2008) Copyright 2008)

(Amici et al. 1989; Stadtman and Berlett 1998; Bedell-Hogan et al. 1993; Requena et al. 2001; Stadtman 2001). Amici et al. (1989) and Requena et al. (2001) found that glutamic semialdehyde was a major product of both oxidized proline and arginine residues. Oxidized proline also produced 2-pyrrolidone (Uchida et al. 1990), hydroxyproline (Stadtman and Levine 2003), and pyroglutamic acid (carbonyl derivatives) (Stadtman and Levine 2003). Pinnell and Martin (1968) and Bedell-Hogan et al. (1993) found that aminoadipic semialdehyde was a carbonyl product of oxidized lysine. Oxidized threonine produces 2-amino-3-ketobutyric acid (Dalle-Donne et al. 2003). Oliver et al. (1987) found that the protein carbonyl derivatives and the loss of enzyme activity were increased with age. An increased carbonyl content of proteins with age and time could be explained by first-order reaction kinetics (Oliver et al. 1987; Carney et al. 1991; Smith et al. 1991; Wells-Knecht et al. 1995). The protein carbonyl derivatives were formed by interaction of proteins with reducing sugars and dicarbonyl compounds or with products of lipid peroxidation. The carbonyl groups were major products of reactive oxygen free radical-mediated oxidation reaction. Carbonyl groups in proteins are widely accepted as an indicator or marker of oxidative protein damage in vivo and in foods (Carney et al. 1991; Stadtman 1992; Dalle-Donne et al. 2003). The presence of carbonyl derivatives of proteins reflects the extent of damage induced

by multiple forms of reactive oxygen species and free radicals as a result of oxidation. The accumulation of proteins with oxidative damage in vivo is associated with ageing, ischemia-reperfusion injury, and a number of age-related diseases including diabetes, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, cataractogenesis, atherosclerosis, Huntington's disease, rheumatoid arthritis, chronic renal failure, and many other disorders (Berlett and Stadtman 1997; Stadtman and Berlett 1998; Butterfield and Kanski 2001; Dalle-Donne et al. 2003).

14.2.3.1 Impact of Oxidation on Proteins

The covalent modification of proteins during ageing as well as food processing has been shown to result in losses of protein functionality or a specific activity of enzymes (Rivett et al. 1985; Meucci et al. 1991); loss of amino acids (Decker et al. 1993); structural, conformational, and functional alteration of amino acids and proteins [e.g., gel strength (rigid gel), elasticity, water holding capacity, gel-forming ability, heat resistance] (Meucci et al. 1991; Decker et al. 1993; Liu et al. 2000; Requena et al. 2001), and lowering of a_w value (Potes et al. 2014). Protein fragmentation (increased carbonyl groups) (Davies 1987; Liu and Xiong 2000; Liu et al. 2000; Lund et al. 2008; Potes et al. 2013), formation of disulfide bonds (Bhoite-Solomon et al. 1992; Liu and Xiong 2000; Liu et al. 2000; Lund et al. 2008; Potes et al. 2013), protein–protein cross-linking (Roubal and Tappel 1966b; Pinnell and Martin 1968; Kanner and Karel 1976; Bhoite-Solomon et al. 1992; Bedell-Hogan et al. 1993; Lund et al. 2008; Potes et al. 2014), decreased protein denaturation (unfolding) temperature (Liu and Xiong 2000; Potes et al. 2013), protein aggregation (Kanner and Karel 1976; Butterfield and Kanski 2001; Potes et al. 2013), increased protein hydrophobicity (Stadtman 2001; Potes et al. 2013), insolubilization and indigestibility of proteins (Roubal and Tappel 1966b; Kanner and Karel 1976; Meucci et al. 1991; Bedell-Hogan et al. 1993; Liu and Xiong 2000; Potes et al. 2013; 2014), polymerization of proteins (Decker et al. 1993; Liu and Xiong 2000; Liu et al. 2000; Potes et al. 2014), and increased surface hydrophobicity of proteins (Meucci et al. 1991; Chao et al. 1997). Decker et al. (1993) demonstrated that oxidative damage to myofibrillar protein in white turkey muscle by iron and copper increased protein carbonyl content, protein hydrophobicity (lower solubility), and loss of myosin and actin proteins with concomitant protein polymerization. Liu et al. (2000) found that the amino acid side chains of whey protein isolate (WPI) and soy protein isolates (SPI) were modified during metal-catalyzed oxidation by $FeCl_3$, H_2O_2, and ascorbate. Such oxidation increased formation of disulfide bonds, protein carbonyls, and free amines in WPI, SPI, and myofibril-protein isolate (1:1) mixtures; and increased elasticity of an SPI gel and interactions of myofibrils with SPI.

Several pathways of protein–protein cross-linking in oxidative modification of proteins were reported by Stadtman and Levine (2003), including: (1) oxidation of cysteine sulfhydryl groups (disulfide bond formation); (2) interaction of carbonyl

groups of oxidized proteins with the amino groups of a lysine residues in the same or different protein molecules; (3) reaction of glycation derived protein carbonyls with lysine or arginine residues in the same or different protein molecules; (4) reaction of dicarbonyl groups (e.g., malondialdehyde and glyoxal) with two lysine residues in the same or different protein molecules; and (5) reaction of amino groups of lysine residues with protein carbonyls produced by lipid peroxidation reactions. The type of cross-linking in the systems was dependent on the oxidizing agents and interaction time (Bhoite-Solomon et al. 1992; Lund et al. 2008). Bhoite-Solomon et al. (1992) found that myoglobin and H_2O_2 (hydroxyl free radicals) induced the formation of intermolecular disulfide cross-links of myosin (protein in heart and skeletal muscle), and myosin formed covalently aggregated protein (intermolecular covalent bonds). The latter aggregates of myosin resulted from pairing of myosin radicals formed by the H_2O_2. Lund et al. (2008) showed that oxidation of myosin by myoglobin and H_2O_2 produced oxidized tyrosine and cysteine residues (thiyl, tyrosyl, and other unidentified radical species) of myosin, and formation of both disulfide linkages and covalent cross-linking. These studies have shown that formation of disulfide bonds and covalent cross-linkages occur in concomitant processes.

14.2.3.2 Control and Prevention of Protein Oxidation

Oxidative damage to proteins is associated with chemical reactions and various environmental factors in vivo and in food processing, which can be governed by following factors:

(i) Use of antioxidants to prevent protein from oxidative damage, by conversion of reactive free radical species to unreactive derivatives.
(ii) Removal of metal ions that can catalyze oxidation such as Fe^{2+}, Fe^{3+}, and Cu^{+} (Decker et al. 1993; Liu et al. 2000; Refsgaard et al. 2000).
(iii) Minimization of irradiation and UV exposure.
(iv) Removal of oxygen, reactive oxygen species, and free radicals.
(v) Control of relevant factors that induce development of protein oxidation, such as glycation or the Maillard reaction and lipid oxidation.

14.2.4 Stability of Carotenoids in Dehydration

When carotenoids are present as crystals, or found within protein complexes of fruits and vegetables, they have low bioavailability (very low adsorption) in the gastrointestinal tract (Williams et al. 1998). Nonetheless, incorporating carotenoids in edible oil can greatly improve their bioavailability during digestion (van Het Hof et al. 2000). A simple method to increase bioavailability of carotenoids in food systems is to incorporate them into the lipid phase of oil-in-water (O/W) emulsions

where the lipid phase is emulsified to a hydrophilic phase with an emulsifier. The emulsion can be combined with a glass-forming hydrophilic component and dehydrated to obtain a continuous phase of dried formulations (Drusch et al. 2006; Ramoneda et al. 2011; Lim et al. 2014; Lim and Roos 2016). Microencapsulation utilizes wall materials to entrap and protect core materials from environmental stresses and provide controlled release of the encapsulated core materials (Shahidi and Han 1993). Spray drying and freeze-drying are commonly used for microencapsulation (Desorby et al. 1997; Ré 1998). Degradation of carotenoids is a typical first-order reaction, and it has been well reported that the loss of carotenoids increased with increasing storage temperatures (Desorby et al. 1997; Henry et al. 1998; Dhuique-Mayer et al. 2007; Hidalgo and Brandolini 2008; Achir et al. 2010; Caliskan et al. 2015). Carotenoid degradation is caused primarily by oxidation, but isomerization during processing may also have significant effects on carotenoid stability (Qiu et al. 2009). The leading causes of isomerization in carotenoids are exposure to light and heat (Liu et al. 2009b; Khoo et al. 2011).

Storage of dehydrated materials above glass transition temperature (T_g) of the glass-forming continuous phase in dried emulsion powders showed reduced rate constants for carotenoids losses (Fig. 14.10). The dynamic process of structural collapse occurred as the result of storage above the T_g at the rate defined by $T - T_g$ (Levi and Karel 1995). Temperatures above the T_g decreased viscosity and increased molecular mobility (Roos and Karel 1991). Structural collapse produced a less porous structure, which reduced oxygen diffusion and affected heat transfer. Harnkasujarit et al. (2012) found that structural collapse reduced the loss of β-carotene in freeze-dried maltodextrin systems. A similar observation was made by Lim et al. (2014) for the loss of lutein and β-carotene in freeze-dried materials with trehalose as the continuous glass-forming matrix. Even though no physical collapse was observed in the study by Lim and Roos (2016) using spray dried emulsions, storing the powders above the T_g led to the formation of a thicker membrane throughout the powder surface as the result of decreasing viscosity and increasing molecular mobility. Structural transformations within powder particles may effectively decrease oxygen permeability and transfer of radicals across interfaces. Dissolved oxygen present within the surface water of the dehydrated matrix decreased with increasing storage temperature as higher storage temperature of up to 100 °C reduced oxygen solubility in water (Hildebrand 1952; Wilhelm et al. 1977). Therefore, lesser amounts of oxygen may diffuse towards the encapsulated carotenoids containing oil at higher storage temperatures, thereby reducing loss of carotenoids. A Maillard reaction may also occur in systems with amino acids and reducing sugars at higher storage temperatures. Melanoidins, having antioxidant capacity, are produced during the reaction, and such NEB products can protect the encapsulated carotenoids against radicals (Wang et al. 2011).

The difference in the matrices obtained from spray drying and freeze-drying also contributed to the difference in carotenoids loss rate. The degradation of carotenoids was faster in freeze-dried emulsions than in spray-dried emulsions (Fig. 14.10). Mahfoudhi and Hamdi (2014, 2015) also reported that the loss of β-carotene was faster in a freeze-dried system than in a spray-dried system. Such differences can be

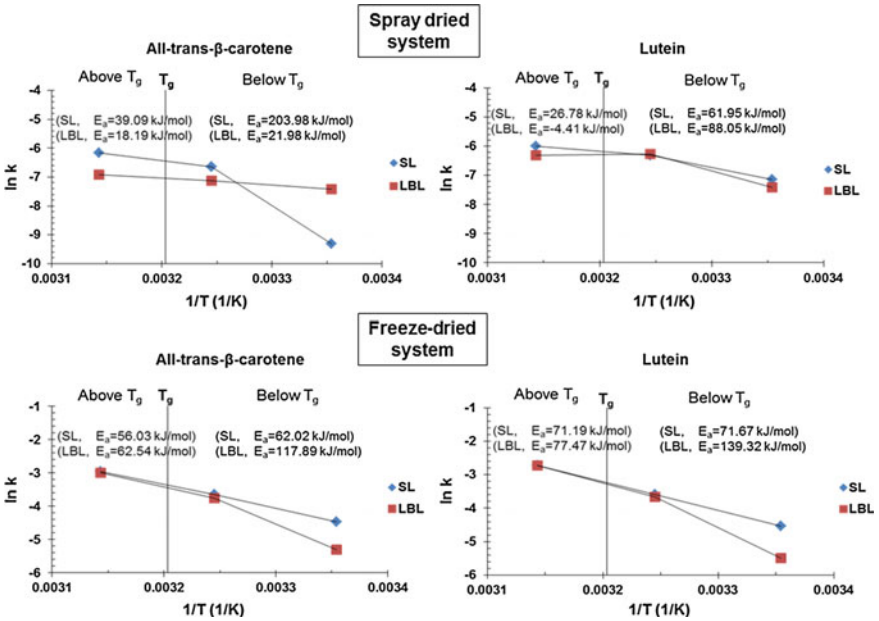

Fig. 14.10 Arrhenius plots of all-*trans*-β-carotene and lutein stored at 25, 35, and 45 °C in humidified (0.33 a_w) spray-dried and freeze-dried single-layer (SL) and layer-by-layer (LBL) emulsions with trehalose and maltodextrin (DE 9-12) as glass formers (Lim and Roos 2016) (Reprinted from Lim and Roos 2016, with permission from Elsevier)

associated with the more porous matrix obtained from freeze-drying. The more porous matrix allows higher rates of oxygen or radicals permeability towards the encapsulated carotenoids containing oil, allowing higher loss of the encapsulated carotenoids. Contrarily, spray drying results in a denser and less porous glass-former matrix, reducing oxygen permeability. The hardening of the outer layer of glass formers followed by thermal expansion of trapped air bubbles in spray drying creates oxygen-containing vacuoles in the powders obtained (Aguilera 1990; Tewa-Tagne et al. 2007). The presence of oxygen within powders results in rapid initial loss kinetics, and subsequent slower loss kinetics commonly observed in spray-dried systems (Fig. 14.11) (Desorby et al. 1999; Lim and Roos 2016). A study by Drusch et al. (2006) also reported a rapid increase of hydroperoxides in fish oil during the first week of storage, followed by a slower increase in the subsequent 8 weeks.

Crystallization of the glass formers in dehydrated matrices resulted in the release of the lipid phase with carotenoids that increased the rate of carotenoids degradation (Buera et al. 2005). Nevertheless, several authors found that the incorporation of high molecular weight carbohydrates with amorphous sugars retarded crystallization of the amorphous sugars (Mazzobre et al. 1997; Gabarra and Hartel 1998; Kouassi and Roos 2001; Potes et al. 2012). The degradation of carotenoids was

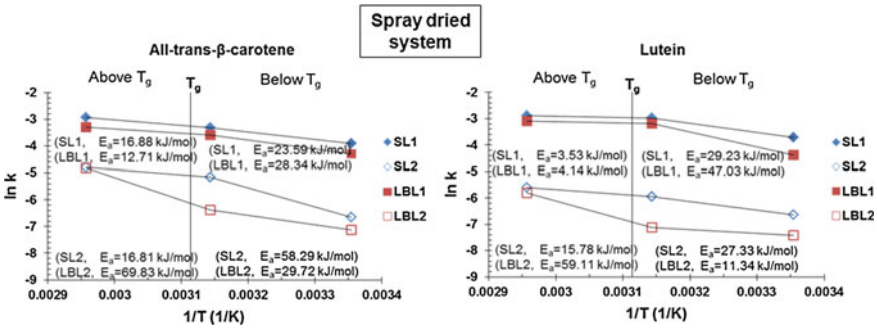

Fig. 14.11 Arrhenius plots of all-*trans*-β-carotene and lutein stored at 25, 45, and 65 °C in spray-dried and freeze-dried single-layer (SL) and layer-by-layer (LBL) emulsions with trehalose and maltodextrin (DE 9-12) as glass-formers obtained as is (~0.25 a_w) from a spray dryer, displaying a rapid first initial first-order loss kinetics (SL1 and LBL1) and subsequent slower first-order loss kinetics (SL2 and LBL2) (Lim and Roos 2016) (Reprinted from Lim and Roos 2016, with permission from Elsevier)

slower in a layer-by-layer (LBL) system than in a single-layer (SL) system (Figs. 14.10 and 14.11). The application of LBL interfacial structures resulted in higher stability of LBL system towards environmental stresses. This was attributed to the denser and thicker interfacial layer of the particles, lower van der Walls attraction strength, and increased steric repulsion of the LBL system (Moreau et al. 2003; Gu et al. 2005; Harnsilawat et al. 2006; Benjamin et al. 2012). a_w of the systems plays a role in the rate of carotenoids degradation. Quast and Karel (1972) reported that the oxidation rate of potato chips decreased with increasing a_w of the systems from 0.01 to 0.4 a_w. Lavelli et al. (2007) also found similar trend in the loss of carotenoids in carrots. Water has inhibitory effect against oxidation as hydrogen bonding of hydroperoxides produced during oxidation inhibits further reactions. Hydration of the trace metals also reduces their catalytic effects. However, both studies found that the reaction rates increased as the a_w of the systems approached values of 0.6 a_w and higher.

Due to the difference in molecular structure, the loss of lutein (xanthophyll) was found to be faster than the loss of β-carotene. The two hydroxyl groups in lutein make lutein more polar and hydrophilic than β-carotene (Farombi and Britton 1999; Updike and Schwartz 2003). This caused lutein to assemble at the oil-WPI interface while the less polar β-carotene was present within the bulk oil. Figure 14.12, captured using Raman-focused ion beam (FIB)-SEM analysis with Raman imaging and scanning electron (RISE) microscopy, shows the location of lutein and β-carotene present within a powder particle of spray-dried emulsion. It can be seen from Fig. 14.12 that lutein and β-carotene were separate within the lipid phase of the spray-dried particle. Lutein occurred at the interfaces of the intact protein-covered oil particles, and it was apparent on the powder particle surfaces. On the other hand, β-carotene was not visible from bulk oil. Lutein at the oil–WPI interface degraded more rapidly as it was exposed to the trapped oxygen within the dehydrated matrix

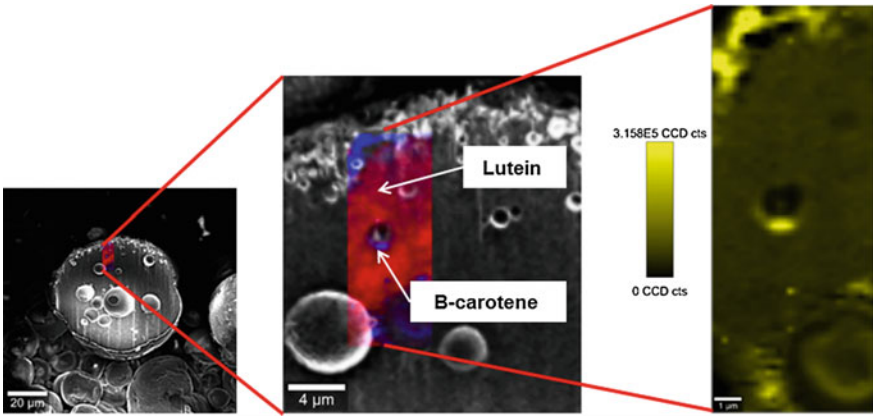

Fig. 14.12 Raman-focused ion beam (FIB)-SEM analysis with Raman imaging and scanning electron (RISE) microscopy images showing locations of lutein (*red*) and β-carotene (*blue*) present within a cut spray-dried particle. The charge-coupled device (ccd) detector scale provides a signal intensity reading for quantification of the carotenoids at the selected area

and possibly to radicals within the hydrophilic phase. The higher polarity of carboxyl groups in fatty acids also caused them to concentrate at the oil–WPI interface, allowing close proximity with the lutein present. As a result, lutein could undergo esterification with fatty acids of the lipid phase of dehydrated emulsions. Reactive oxygen groups and free radicals formed from lipid oxidation could attack the carotenoids present and increase their degradation rate. Lipid oxidation may also affect the colour of the systems due to bleaching of carotenoids and browning (Venolia and Tappel 1958; Zirlin and Karel 1969). Oil-soluble bioactive components, such as carotenoids, are commonly used in the food and pharmaceutical industries for medical foods, nutritional supplements, and infant formulas. Therefore, it is crucial to acknowledge and be aware of the factors affecting chemical stability of bioactive components to prevent instability and high rates of degradation.

14.3 Conclusion

The browning and oxidation reactions of carbohydrate, protein, and lipid components during processing and storage of high-solids systems exert both positive and negative effects on physical, physicochemical, sensorial, thermal, and molecular properties, including the loss of nutrients, bioactive and heat-sensitive compounds (e.g., vitamins, carotenoids and other antioxidants, amino acids, and proteins), sensory and physical properties (e.g., formation of desired and undesired colour and flavour, modifications to structure, softened and hardened texture), and functional properties (solubility, digestibility, heat and pH sensitivity, polymerization, cross-linking of proteins via covalent and disulfide bonds). Such complex

phenomena are important factors that must be considered and controlled during processing and storage of high-solids food systems and pharmaceutical formulations. Recognizing factors affecting chemical stability of bioactive components may help to avoid instability and high degradation rates. Furthermore, consequences of chemical changes resulting from reactions, particularly oxidative and free radical-related, between components in food products have been found to be risk factors for degenerative diseases and accelerated ageing.

References

Acevedo N, Schebor C, Buera MP (2006) Water–solids interactions, matrix structural properties and the rate of non-enzymatic browning. J Food Eng 108:1108–1115

Acevedo N, Schebor C, Buera MP (2008) Non-enzymatic browning kinetics analysed through water–solid interactions and water mobility in dehydrated potato. Food Chem 108:900–906

Achir N, Randrianatoandro VA, Bohuon P, Laffargue A, Avallone S (2010) Kinetic study of β-carotene and lutein degradation in oils during heat treatment. Eur J Lipid Sci Tech 112:349–361

Addis PB (1986) Occurrence of lipid oxidation products in foods. Food Chem Toxic 24:1021–1030

Aguilera JM (1990) Microstructural principles of food processing and engineering. Elsevier, London, pp 309–327

Ahmed MU, Thorpe SR, Baynes JW (1986) Identification of N^{ε}-carboxylethyllysine as a degradation product of fructoselysine in glycated protein. J Biol Chem 261:4889–4894

Ajandouz EH, Puigserver A (1999) Nonenzymatic browning reaction of essential amino acids: effect of pH on caramelization and Maillard reaction kinetics. J Agric Food Chem 47:1786–1793

Ajandouz EH, Tchiakpe LS, Dalle Ore F, Benajiba A, Puigserver A (2001) Effects of pH on caramelization and Maillard reaction kinetics in fructose-lysine model systems. J Food Sci 66:926–931

Akhtar M, Dickinson E (2007) Whey protein–maltodextrin conjugates as emulsifying agents: an alternative to gum arabic. Food Hydrocoll 21:607–616

Allison SD, Chang B, Randolph TW, Carpenter JF (1999) Hydrogen bonding between sugar and protein is responsible for inhibition of dehydration-induced protein unfolding. Arch Biochem Biophys 365:289–298

Ames JM (2001) Melanoidins as pro- or antioxidants. Cerevisia 26:210–216

Amici A, Levine RL, Tsai L, Stadtman ER (1989) Conversion of amino acid residues in proteins and amino acid homopolymers to carbonyl derivatives by metal-catalyzed oxidation reactions. J Biol Chem 264:3341–3346

Anderson MM, Hazen SL, Hsu FF, Heinecke JW (1997) Human neutrophils employ the myeloperoxidase-hydrogen peroxide-chloride system to convert hydroxyl-amino acids into glycolaldehyde, 2-hydroxypropanol, and acrolein. J Clin Invest 99:424–432

Arakawa T, Timasheff SN (1982) Stabilization of protein structure by sugars. Biochemistry 21:6536–6544

Ashoor SH, Zent JB (1984) Maillard browning of common amino acids and sugars. J Food Sci 49:1206–1207

Back JF, Oakenfull D, Smith MB (1979) Increased thermal stability of proteins in the presence of sugars and polyols. Biochemistry 18:5191–5196

Balasundram N, Sundram K, Samman S (2006) Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem 99:191–203

Ball CD, Hardt CR, Duddles WJ (1943) The influence of sugars on the formation of sulfhydryl groups in heat denaturation and heat coagulation of egg albumin. J Biol Chem 151:163–169
Baskol G, Demir H, Baskol M, Kilic E, Ates F, Karakukcu C, Ustdal M (2006) Investigation of protein oxidation and lipid peroxidation in patients with rheumatoid arthritis. Cell Biochem Funct 24:307–311
Baxter JH (1995) Free amino acid stability in reducing sugar systems. J Food Sci 60:405–408
Bedell-Hogan D, Trackman P, Abrams W, Rosenbloom J, Kagan H (1993) Oxidation, cross-linking, and insolubilization of recombinant tropoelastin by purified lysyl oxidase. J Biol Chem 268:10345–10350
Beisswenger PJ, Moore LL, Brinck-Johnsen T, Curphey TJ (1993) Increased collagen-linked pentosidine levels and advanced glycosylation end products in early diabetic nephropathy. J Clin Invest 92:212–217
Bell LN (1996) Kinetics of non-enzymatic browning in amorphous solids systems: distinguishing the effects of water activity and the glass transition. Food Res Int 28:591–597
Bell LN (1997) Maillard reaction as influenced by buffer type and concentration. Food Chem 59:143–147
Benjakul S, Visessanguan W, Phongkanpai V, Tanaka M (2005) Antioxidative activity of caramelisation products and their preventive effect on lipid oxidation in fish mince. Food Chem 90:231–239
Benjamin O, Silcock P, Leus M, Everett DW (2012) Multilayer emulsions as delivery systems for controlled release of volatile compounds using pH and salt triggers. Food Hydrocoll 27:109–118
Berlett BS, Stadtman ER (1997) Protein oxidation in aging, disease, and oxidative stress. J Biol Chem 272:20313–20316
Bhoit-Solomon V, Kessler-Icekson G, Shaklai N (1992) Peroxidative crosslinking of myosins. Biochem Int 26:181–189
Boye JI, Alli I (2000) Thermal denaturation of mixtures of α-lactalbumin and β-lactoglobulin: a differential scanning calorimetric study. Food Res Int 33:673–682
Breitling-Utzmann CM, Unger A, Friedl DA, Lederer MO (2001) Identification and quantification of phosphatidylethanolamine-derived glucosylamines and aminoketoses from human erythrocytes-influence of glycation products on lipid peroxidation. Arch Biochem Biophys 391:245–254
Briganti S, Picardo M (2003) Antioxidant activity, lipid peroxidation and skin diseases. What's new? J Eur Acad Dermatol Venereol 17:663–669
Bucala R, Makita Z, Koschinsky T, Cerami A, Vlassara H (1993) Lipid advanced glycosylation: pathway of lipid oxidation in *vivo*. Proc Natl Acad Sci U S A 90:6434–6438
Buera MP, Karel M (1995) Effect of physical changes on the rates of nonenzymatic browning and related reactions. Food Chem 52:167–173
Buera P, Schebor C, Elizalde B (2005) Effects of carbohydrate crystallization on stability of dehydrated foods and ingredient formulations. J Food Eng 67:157–165
Bunn HF, Higgins PJ (1981) Reaction of monosaccharides with proteins: possible evolutionary significances. Science 213:222–224
Burin L, Jouppila K, Roos YH, Kansikas J, Buera MP (2004) Retention of β-galactosidase activity as related to Maillard reaction, lactose crystallization, collapse and glass transition in low moisture whey systems. Int Dairy J 14:517–525
Butterfield DA, Kanski J (2001) Brain protein oxidation in age-related neurodegenerative disorders that are associated with aggregated proteins. Mech Ageing Dev 122:945–962
Caliskan G, Lim ASL, Roos YH (2015) Beta-carotene stability in extruded snacks produced using interface engineered emulsions. Int J Food Prop 18:2256–2267
Carney JM, Starke-Reed PE, Oliver CN, Landum RW, Cheng MS, Wu JF, Floyd RA (1991) Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity, and loss in temporal and spatial memory by chronic administration of the spin-trapping compound *N*-tert-butyl-alpha-phenylnitrone. Proc Natl Acad Sci U S A 88:3633–3636

Carpenter JF, Crowe JH (1989) An infrared spectroscopic study of the interactions of carbohydrates with dried proteins. Biochemistry 28:3916–3922
Carpenter JF, Crowe LM, Crowe JH (1987) Stabilization of phosphofructokinase with sugars during freeze-drying: characterization of enhanced protection in the presence of divalent cations. Biochim Biophys Acta 923:109–115
Cervato G, Cazzola R, Cestaro B (1999) Studies on the antioxidant activity of milk caseins. Int J Food Sci Nutr 50:291–296
Chao C-C, Ma Y-S, Stadtman ER (1997) Modification of protein surface hydrophobicity and methionine oxidation by oxidative systems. Proc Natl Acad Sci U S A 94:2969–2974
Chen S-L, Yang D-J, Chen H-Y, Liu S-C (2009) Effect of hot acidic fructose solution on caramelisation intermediates including colour, hydroxymethylfurfural and antioxidative activity changes. Food Chem 114:582–588
Chen Y-J, Liang L, Liu X-M, Labuza TP, Zhou P (2012) Effect of fructose and glucose on glycation of β-lactoglobulin in an intermediate-moisture food model system: analysis by liquid chromatography-mass spectrometry (LC-MS) and data-independent acquisition LC-MS ($LC\text{-}MS^E$). J Agric Food Chem 60:10674–10682
Chio KS, Tappel AL (1969) Inactivation of ribonuclease and other enzymes by peroxidizing lipids and by malonaldehyde. Biochemistry 8:2827–2832
Coenen JWE (1976) Hydrogenation of edible oils. J Am Oil Chemists' Soc 53:382–389
Colaco CA, Roser BJ (1994) Atherosclerosis and glycation. BioEssays 16:145–147
Colbert LB, Decker EA (1991) Antioxidant activity of an ultrafiltration permeate from acid whey. J Food Sci 56:1248–1250
Creeth JM, Cooper B, Donald AS, Clamp JR (1983) Studies of the limited degradation of mucus glycoproteins. The effect of dilute hydrogen peroxide. Biochem J 211:323–332
Cucu T, Devreese B, Mestdagh F, Kerkaert B, Meulenaer BD (2011) Protein–lipid interactions during the incubation of whey proteins with autoxidizing lipids. Int Dairy J 21:427–433
Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R (2003) Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta 329:23–38
Davies KJA (1987) Protein damage and degradation by oxygen radicals. J Biol Chem 262:9895–9901
Decker EA, Welch B (1990) Role of ferritin as a lipid oxidation catalyst in muscle food. J Agric Food Chem 38:674–677
Decker EA, Xiong YL, Calvert JT, Crum AD, Blanchard SP (1993) Chemical, physical, and functional properties of oxidized turkey white muscle myofibrillar proteins. J Agric Food Chem 41:186–189
Degenhardt TP, Grass L, Reddy S, Thorpe SR, Diamandis EP, Baynes JW (1997) The serum concentration of the advanced glycation end product N^{ε}-(carboxymethyl)lysine is increased in uremia. Kidney Int 52:1064–1067
Desobry SA, Netto FM, Labuza TP (1999) Influence of maltodextrin systems atan equivalent 25DE on encapsulated β-carotene loss during storage. J Food Process Preserv 23:39–55
Desorbry SA, Netto FM, Labuza TP (1997) Comparison of spray-drying, drum drying and freeze-drying for β-carotene encapsulation and preservation. J Food Sci 62:1158–1162
Dhuique-Mayer C, Tbatou M, Carail M, Caris-Veyrat C, Dornier M, Amiot MJ (2007) Thermal degradation of antioxidant micronutrients in citrus juice: kinetics and newly formed compounds. J Agric Food Chem 55:4209–4216
Dills WL (1993) Protein fructosylation: fructose and the Maillard reaction. Am J Clin Nutr 58:779S–787S
Drusch S, Serfert Y, Van Den Heuvel A, Schwarz K (2006) Physicochemical characterization and oxidative stability of fish oil encapsulated in an amorphous matrix containing trehalose. Food Res Int 39:807–815
Dyer DG, Blackledge JA, Thorpe SR, Baynes JW (1991) Formation of pentosidine during nonenzymatic browning of proteins by glucose. Identification of glucose and other carbohydrates as possible precursors of pentosidine in vivo. J Biol Chem 266:11654–11660

Dyer DG, Dunn JA, Thorpe SR, Bailie KE, Lyons TJ, McCance DR, Baynes JW (1993) Accumulation of Maillard reaction products in skin collagen in diabetes and aging. J Clin Invest 91:2463–2469

Easa AM, Hill SE, Mitchell JR, Taylor AJ (1996) Bovine serum albumin gelation as a result of the Maillard reaction. Food Hydrocoll 10:199–202

Edelman R, Kusner I, Kisiliak R, Srebnik S, Livney YD (2015) Sugar stereochemistry effects on water structure and on protein stability: the templating concept. Food Hydrocoll 48:27–37

Emken EA (1984) Nutrition and biochemistry of trans and positional fatty acid isomers in hydrogenated oils. Ann Rev Nutr 4:339–376

Farombi EO, Britton G (1999) Antioxidant activity of palm oil carotenes in organic solution: effects of structure and chemical reactivity. Food Chem 64:315–321

Fox M, Loncin M, Weiss M (1983) Investigations into the influence of water activity, pH and heat treatment on the velocity of the Maillard reaction in foods. J Food Qual 6:103–118

Fry LK, Stegink LD (1982) Formation of Maillard reaction products in parenteral alimentation solutions. J Nutr 112:1631–1637

Fu M-X, Knecht KJ, Thorpe SR, Baynes JW (1992) Role of oxygen in cross-linking and chemical modification of collagen by glucose. Diabetes 41:42–48

Funes JA, Weiss U, Karel M (1982) Effects of reaction conditions and reactant concentrations on polymerization of lysozyme reacted with peroxidizing lipids. J Agric Food Chem 30:1204–1208

Gabarra P, Hartel RW (1998) Corn syrup solids and their saccharide fractions affect crystallization of amorphous sucrose. J Food Sci 63:523–528

Garrison WM (1987) Reaction mechanisms in the radiolysis of peptides, polypeptides, and proteins. Chem Rev 87:381–398

Gebicki S, Gebicki JM (1993) Formation of peroxides in amino acids and proteins exposed to oxygen free radicals. Biochem J 289:743–749

Ghanem AA, Elewa A, Arafa LF (2011) Pentosidine and *N*-carboxymethyl-lysine: Biomarkers for type 2 diabetic retinopathy. Eur J Opthalmol 21:48–54

Gökmen V, Şenyuva HZ (2007) Acrylamide formation is prevented by divalent cations during the Maillard reaction. Food Chem 103:196–203

Gomyo T, Horikoshi M (1976) On the interaction of melanoidin with metallic ions. Agric Biol Chem 40:33–40

Grandhee SK, Monnier VM (1991) Mechanism of formation of the Maillard protein cross-link pentosidine. J Biol Chem 266:11649–11653

Greene BE (1969) Lipid oxidation and pigment changes in raw beef. J Food Sci 34:110–113

Gu YS, Decker EA, McClements DJ (2005) Production and characterization of oil-in-water emulsions containing droplets stabilized by multilayer membranes consisting of β-lactoglobulin, ι-carrageenan and gelatin. Langmuir 21:5752–5760

Gutteridge JMC, Paterson SK, Segal AW, Halliwell B (1981) Inhibition of lipid peroxidation by the iron-binding protein lactoferrin. Biochem J 199:259–261

Handa A, Kuroda N (1999) Functional improvements in dried egg white through Maillard reaction. J Agric Food Chem 47:1845–1850

Harnkarnsujarit N, Charoenrein S, Roos YH (2012) Reversed phase HPLC analysis of stability and microstructural effects on degradation kinetics of β-carotene encapsulated in freeze-dried maltodextrin–emulsion systems. J Agric Food Chem 60:9711–9718

Harnsilawat T, Pongsawatmanit R, McClements DJ (2006) Influence of pH and ionic strength on formation and stability of emulsions containing oil droplets coated by β-lactoglobulin–alginate interfaces. Biomacromolecules 7:2052–2058

Hayase F, Nagarai RH, Miyata S, Njoroge FG, Monnier VM (1989) Aging of proteins: immunological detection of a glucose-derived pyrrole formed during Maillard reaction in vivo. J Biol Chem 263:3758–3764

Hayase F, Shibuya T, Sato J, Yamamoto M (1996) Effects of oxygen and transition metals on the advanced Maillard reaction of proteins with glucose. Biosci Biotech Biochem 60:1820–1825

Henry LK, Catignani GL, Schwartz SJ (1998) Oxidative degradation kinetics of lycopene, lutein, and 9-cis and all-trans-carotene. J Am Oil Chem Soc 75:823–829
Hidalgo A, Brandolini A (2008) Kinetics of carotenoids degradation during the storage of einkorn (*Triticum monococcum* L. ssp. *monococcum*) and bread wheat (*Triticum aestivum* L. ssp. *aestivum*) flours. J Agric Food Chem 56:11300–11305
Hidalgo FJ, Kinsella JE (1989) Changes induced in β-lactoglobulin B following interactions with linoleic acid 13-hydroperoxide. J Agric Food Chem 37:860–866
Hidalgo FJ, Zamora R (2000) The role of lipids in nonenzymatic browning. Grasas Aceites 51:35–49
Hildebrand JH (1952) The temperature dependence of the solubility of solid nonelectrolytes. J Chem Phys 20:190–191
Hirata K, Kubo K (2004) Relationship between blood levels of *N*-carboxymethyl-lysine and pentosidine and the severity of microangiopathy in type 2 diabetes. Endocr J 51:537–544
Hodge JE (1953) Dehydrated foods, chemistry of browning reactions in model systems. J Agric Food Chem 1:928–943
Horiuchi S (1996) Advanced glycation end products (AGE)-modified proteins and their potential relevance to atherosclerosis. Trends Cardiovasc Med 6:163–168
Hu M, McClements DJ, Decker EA (2003) Lipid oxidation in corn oil-in-water emulsions stabilized by casein, whey protein isolate, and soy protein isolate. J Agric Food Chem 51:1696–1700
Jakobsen M, Bertelsen G (2000) Colour stability and lipid oxidation of fresh beef. Development of a response surface model for predicting the effects of temperature, storage time, and modified atmosphere composition. Meat Sci 54:49–57
Jones LS, Kaufmann A, Middaugh CR (2005) Silicone oil induced aggregation of proteins. J Pharm Sci 94:918–927
Kanner J, Karel M (1976) Changes in lysozyme due to reactions with peroxidizing methyl linoleate in a dehydrated model system. J Agric Food Chem 24:468–472
Karel M (1973) Protein–lipid interactions. J Food Sci 38:756–763
Karel M, Schaich K, Roy RB (1975) Interaction of peroxidizing methyl linoleate with some proteins and amino acids. J Agric Food Chem 23:159–163
Karmas R, Buera MP, Karel M (1992) Effect of glass transition on rates of nonenzymatic browning in food systems. J Agric Food Chem 40:873–879
Kato Y, Watanabe K, Sato Y (1981) Effect of Maillard reaction on some physical properties of ovalbumin. J Food Sci 46:1835–1839
Kato Y, Matsuda T, Kato N, Watanabe K, Nakamura R (1986) Browning and insolubilization of ovalbumin by the Maillard reaction with some aldohexoses. J Agric Food Chem 34:351–355
Kato H, Cho RK, Okitani A, Hayase F (1987) Responsibility of 3-deoxyglucosone for the glucose-induced polymerization of proteins. J Agric Biol Chem 51:683–689
Kato Y, Matsuda T, Kato N, Nakamura R (1989) Maillard reaction of disaccharides with protein: suppressive effect of nonreducing end pyranoside groups on browning and protein polymerization. J Agric Food Chem 37:1077–1081
Kella NKD, Poola I (1985) Sugars decrease the thermal denaturation and aggregation of arachin. Int J Pept Protein Res 26:390–399
Khoo HE, Prasad KN, Kong KW, Jiang Y, Ismail A (2011) Carotenoids and their isomers: color pigments in fruits and vegetables. Molecules 16:1710–1738
Kinsella JE, Whitehead DM (1989) Proteins in whey: chemical, physical, and functional properties. Adv Food Nutr Res 33:343–438
Kirigaya N, Kato H, Fujimaki M (1968) Studies on antioxidant activity of nonenzymatic browning reaction products. Agric Biol Chem 32:287–290
Koch RB (1962) Dehydrated foods and model systems. In Symposium on Foods: Lipids and Their Oxidation. Shultz HW, Day EA, Sinnhuber RO Eds., AVI, Westport, Connecticut, USA, Chapter 13, pp. 230–251
Kouassi K, Roos YH (2001) Glass transition and water effects on sucrose inversion in non-crystalline carbohydrate food systems. Food Res Int 34:895–901

Krokida MK, Oreopoulou V, Maroulis ZB (2000) Water loss and oil uptake as a function of frying time. J Food Eng 44:39–46
Kwak E-J, Lim S-I (2004) The effect of sugar, amino acid, metal ion, and NaCl on model Maillard reaction under pH control. Amino Acids 27:85–90
Labuza TP, Dugan LR Jr (1971) Kinetics of lipid oxidation in foods. CRC Crit Rev Food Technol 2:355–405
Labuza TP, Saltmarch M (1981) Kinetics of browning and protein quality loss in whey powders during steady state and nonsteady state storage conditions. J Food Sci 47:92–96
Labuza TP, Tsuyuki H, Karel M (1969) Kinetics of linoleate oxidation in model systems. J Am Oil Chem Soc 46:409–416
Labuza TP, McNally L, Gallagher D, Hawkes J, Hurtado F (1972) Stability of intermediate moisture foods. 1. Lipid oxidation. J Food Sci 37:154–159
Lavelli V, Zanoni B, Zaniboni A (2007) Effect of water activity on carotenoid degradation in dehydrated carrots. Food Chem 104:1705–1711
Leake L, Karel M (1982) Polymerization and denaturation of lysozyme exposed to peroxidizing lipids. J Food Sci 47:737–739
Levi G, Karel M (1995) Volumetric shrinkage (collapse) in freeze-dried carbohydrates above their glass transition temperature. Food Res Int 28:145–151
Lewis VM, Lea CH (1950) A note on the relative rates of reaction of several reducing sugars and sugar derivatives with casein. Biochim Biophys Acta 4:532–534
Lievonen SM, Laaksonen TJ, Roos YH (1998) Glass transition and reaction rates: nonenzymatic browning in glassy and liquid systems. J Agric Food Chem 46:2778–2784
Liggins J, Furth AJ (1997) Role of protein-bound carbonyl groups in the formation of advanced glycation endproducts. Biochim Biophys Acta 1361:123–130
Lim ASL, Roos YH (2016) Spray drying of high hydrophilic solids emulsions with layered interface and trehalose-maltodextrin as glass formers for carotenoids stabilization. J Food Eng 171:174–184
Lim ASL, Griffin C, Roos YH (2014) Stability and loss kinetics of lutein and β-carotene encapsulated in freeze-dried emulsions with layered interface and trehalose as glass former. Food Res Int 62:403–409
Liu G, Xiong YL (2000) Electrophoretic pattern, thermal denaturation, and in vitro digestibility of oxidized myosin. J Agric Food Chem 48:624–630
Liu G, Xiong YL, Butterfield DA (2000) Chemical, physical, and gel-forming properties of oxidized myofibrils and whey- and soy-protein isolates. J Food Sci 65:811–818
Liu SC, Lin JT, Yang DJ (2009a) Determination of cis- and trans-α- and β-carotenoids in taiwanese sweet potatoes (*Ipomoea batatas* (L.) Lam.) harvested at various times. Food Chem 116:605–610
Liu X, Zhou P, Tran A, Labuza TP (2009b) Effects of polyols on the stability of whey proteins in intermediate-moisture food model systems. J Agric Food Chem 57:2339–2345
Loveday SM, Hindmarsh JP, Creamer LK, Singh H (2009) Physicochemical changes in a model protein bar during storage. Food Res Int 42:798–806
Lund MN, Luxford C, Skibsted LH, Davies MJ (2008) Oxidation of myosin by haem proteins generates myosin radicals and protein cross-links. Biochem J 410:565–574
Lyons TJ (1993) Glycation and oxidation: a role in the pathogenesis of atherosclerosis. Am J Cardiol 71:26–31
Mahfoudhi N, Hamdi S (2014) Kinetic degradation and storage stability of β-carotene encapsulated by spray drying using almond gum and gum arabic as wall materials. J Polym Eng 34:683–693
Mahfoudhi N, Hamdi S (2015) Kinetic degradation and storage stability of β-carotene encapsulated by freeze-drying using almond gum and gum arabic as wall materials. J Food Process Preserv 39:896–906
Makita Z, Radoff S, Rayfield EJ, Yang Z, Skolnik E, Delaney V, Friedman EA, Cerami A, Vlassara H (1991) Advanced glycosylation end products in patients with diabetic nephropathy. N Engl J Med 325:836–842

Makris DP, Rossiter JT (2000) Heat-induced, metal-catalyzed oxidative degradation of quercetin and rutin (quercetin 3-*o*-rhamnosylglucoside) in aqueous model systems. J Agric Food Chem 48:3830–3838

Markesbery WR, Lovell MA (1998) Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease. Neurobiol Aging 19:33–36

Mastrocola D, Munari M (2000) Progress of the Maillard reaction and antioxidant action of Maillard reaction products in preheated model systems during storage. J Agric Food Chem 48:3555–3559

Mastrocola D, Munari M, Cioroi M, Lerici CR (2000) Interaction between Maillard reaction products and lipid oxidation in starch-based model systems. J Sci Food Agric 80:684–690

Mazzobre MF, Buera MP, Chirife J (1997) Protective role of trehalose on thermal stability of lactase in relation to its glass and crystal forming properties and effect of delaying crystallization. Lebensm Wiss Technol 30:324–329

McCance DR, Dyer DG, Dunn JA, Bailie KE, Thorpe SR, Baynes JW, Lyons TJ (1993) Maillard reaction products and their relation to complications in insulin-dependent diabetes mellitus. J Clin Invest 91:2470–2478

McPherson JD, Shilton BH, Walton DJ (1988) Role of fructose in glycation and cross-linking of proteins. Biochemistry 27:1901–1907

Meucci E, Mordente A, Martorana GE (1991) Metal-catalyzed oxidation of human serum albumin: conformational and functional changes. Implications in protein aging. J Biol Chem 266:4692–4699

Miyata T, Ueda Y, Yamada Y, Izuhara Y, Wada T, Jadoul M, Saito A, Kurokawa K, van Ypersele de Strihou C (1998) Accumulation of carbonyls accelerates the formation of pentosidine, an advanced glycation end product: carbonyl stress in uremia. J Am Soc Nephrol 9:2349–2356

Molnar-Perl I, Friedman M (1990) Inhibition of browning by sulfur amino acids. 2. Fruit juices and protein-containing foods. J Agric Food Chem 38:1648–1651

Monnier VM (1990) Nonenzymatic glycosylation, the Maillard reaction and the aging process. J Gerontol 45:105–111

Morcira RG, Sun X, Chen Y (1997) Factors affecting oil uptake in tortilla chips in deep-fat frying. J Food Eng 31:485–498

Moreau L, Kim H-J, Decker EA, McClements DJ (2003) Production and characterization of oil-in-water emulsions containing droplets stabilized by β-lactoglobulin–pectin membranes. J Agric Food Chem 51:6612–6617

Morita J, Kashimura N (1991) The Maillard reaction of DNA with D-fructose 6-phosphate. Agric Biol Chem 55:1359–1366

Mulvihill DM, Donovan M (1987) Whey proteins and their thermal denaturation—a review. Ir J Food Sci Technol 11:43–75

Mulvihill DM, Kinsella JE (1987) Gelation characteristics of whey proteins and beta-lactoglobulin. Food Technol. 41:102–111

Negre-Salvayre A, Coatrieux C, Ingueneau C, Salvayre R (2008) Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. Br J Pharmacol 153:6–20

Nielsen HK, Löliger J, Hurrell RF (1985) Reactions of proteins with oxidizing lipids. Br J Nutr 53:61–73

Nursten HE (1981) Recent developments in studies of the Maillard reaction. Food Chem 6:263–277

Oliver CN, Ahn B-W, Moerman EJ, Goldstein S, Stadtman ER (1987) Age-related changes in oxidized proteins. J Biol Chem 262:5488–5491

Ordonez JA, Ledward DA (1977) Lipid and myoglobin oxidation in pork stored in oxygen- and carbon dioxide-enriched atmospheres. Meat Sci 1:41–48

Ortwerth BJ, Olesen PR (1988) Ascorbic acid-induced crosslinking of lens proteins: evidence supporting a Maillard reaction. Biochim Biophys Acta 956:10–22

Öste RE, Dahlqvist A, Sjöström H, Norén O, Miller R (1986) Effect of Maillard reaction products on protein digestion. In vitro studies. J Agric Food Chem 34:355–358

Peña-Ramos EA, Xiong YL (2003) Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. Meat Sci 64:259–263

Pilková L, Pokorný J, Davídek J (1990) Browning reactions of Heyns rearrangement products. Nahrung 34:759–764

Pinnell SR, Martin GR (1968) The cross-linking of collagen and elastin: enzymatic conversion of lysine in peptide linkage to α-aminoadipic-δ-semialdehyde (allysine) by an extract from bone. Proc Natl Acad Sci U S A 61:708–716

Pinthus EJ, Weinberg P, Saguy IS (1995) Oil uptake in deep fat frying as affected by porosity. J Food Sci 60:767–769

Plachta H, Bartnikowska E, Obara A (1992) Lipid peroxides in blood from patients with atherosclerosis of coronary and peripheral arteries. Clin Chem Acta 211:101–112

Pomeranz Y, Johnson JA, Shellenberger JA (1962) Effect of various sugars on browning. J Food Sci 27:350–354

Potes N, Kerry JP, Roos YH (2012) Additivity of water sorption, alpha-relaxations and crystallization inhibition in lactose–maltodextrin systems. Carbohyd Polym 89:1050–1059

Potes N, Kerry JP, Roos YH (2013) Oil as reaction medium for glycation, oxidation, denaturation, and aggregation of whey protein systems of low water activity. J Agric Food Chem 61:3748–3756

Potes N, Kerry JP, Roos YH (2014) Protein modifications in high protein–oil and protein–oil–sugar systems at low water activity. Food Biophys 9:49–60

Qiu D, Chen ZH, Li HR (2009) Effect of heating on solid β-carotene. Food Chem 112:344–349

Quast DG, Karel M (1972) Effects of environmental factors on the oxidation of potato chips. J Food Sci 37:584–588

Ramoneda XA, Ponce-Cevallos PA, Buera MP, Elizalde BE (2011) Degradation of β-carotene in amorphous polymer matrices. Effect of water sorption properties and physical state. J Sci Food Agr 91:2587–2593

Ré MI (1998) Microencapsulation by spray-drying. Dry Technol 16:1195–1236

Reddy S, Bichler J, Wells-Knecht KJ, Thorpe SR, Baynes JW (1995) N^{ε}-(carboxymethyl)lysine is a dominant advanced glycation end product (AGE) antigen in tissue proteins. Biochemistry 34:10872–10878

Refsgaard HHF, Tsai T, Stadtman ER (2000) Modifications of proteins by polyunsaturated fatty acid peroxidation products. Proc Natl Acad Sci U S A 97:611–616

Remig V, Franklin B, Margolis S, Kostas G, Nece T, Street JC (2010) Trans fats in America: a review of their use, consumption, health implications, and regulation. J Am Diet Assoc 110:585–592

Requena JR, Fu M-X, Ahmed MU, Jenkins AJ, Lyons TJ, Thorpe SR (1996) Lipoxidation products as biomarkers of oxidative damage to proteins during lipid peroxidation reactions. Nephrol Dial Transplant 11:48–53

Requena JR, Chao C-C, Levine RL, Stadtman ER (2001) Glutamic and aminoadipic semialdehydes are the main carbonyl products of metal-catalyzed oxidation of proteins. Proc Natl Acad Sci U S A 98:69–74

Rhee KS, Ziprin YA, Ordonez G (1987) Catalysis of lipid oxidation in raw and cooked beef by metmyoglobin-H_2O_2, nonheme iron, and enzyme systems. J Agric Food Chem 35:1013–1017

Rice-Evans CA, Miller NJ, Paganga G (1997) Antioxidant properties of phenolic compounds. Trends Plant Sci 2:152–159

Rich LM, Foegeding EA (2000) Effects of sugars on whey protein isolate gelation. J Agric Food Chem 48:5046–5052

Richards MP, Hultin HO (2000) Effect of pH on lipid oxidation using trout hemolysate as a catalyst: a possible role for deoxyhemoglobin. J Agric Food Chem 48:3141–3147

Rivett AJ, Roseman JE, Oliver CN, Levine RL, Stadtman ER (1985) Covalent modification of proteins by mixed-function oxidation: recognition by intracellular proteases. Prog Clin Biol Res 180:317–328

Roos YH (1993) Water activity and physical state effects on amorphous food stability. J Food Process Preserv 16:433–447

Roos YH (1995a) Glass transition-related physicochemical changes in foods. Food Technol 49:97–102
Roos YH (1995b) Phase transitions in foods. Academic Press Inc, California
Roos Y, Karel M (1991) Plasticizing effect of water on thermal behavior and crystallization of amorphous food models. J Food Sci 56:38–43
Roos YH, Jouppila K, Zielasko B (1996a) Non-enzymatic browning-induced water plasticization. J Therm Anal 47:1437–1450
Roos Y, Karel M, Kokini JL (1996b) Glass transitions in low moisture and frozen foods: effects on shelf life and quality. Food Technol. 50:95–139
Rouach H, Fataccioli V, Gentil M, French SW, Morimoto M, Nordmann R (1997) Effect of chronic ethanol feeding on lipid peroxidation and protein oxidation in relation to liver pathology. Hepatology 25:351–355
Roubal WT (1971) Free radicals, malonaldehyde and protein damage in lipid-protein systems. Lipids 6:62–64
Roubal WT, Tappel AL (1966a) Damage to proteins, enzymes, and amino acids by peroxidizing lipids. Arch Biochem Biophys 113:5–8
Roubal WT, Tappel AL (1966b) Polymerization of proteins induced by free radical lipid peroxidation. Arch Biochem Biophys 113:150–155
Sakanaka S, Tachibana Y, Ishihara N, Juneja LR (2004) Antioxidant activity of egg-yolk hydrolysates in a linoleic acid oxidation system. Food Chem 86:99–103
Salman AG, Mansour DE, Swelem AH, AI-Zawahary WM, Radwan AA (2009) Pentosidine—a new biochemical marker in diabetic retinopathy. Ophthalmic Res 42:96–98
Sasaki N, Fukatsu R, Tsuzuki K, Hayashi Y, Yoshida T, Fujii N, Koike T, Wakayama I, Yanagihara R, Garruto R, Amano N, Makita Z (1998) Advanced glycation end products in Alzheimer's disease and other neurodegenerative diseases. Am J Pathol 153:1149–1155
Sattar A, deMan JM, Furia TE (1975) Photooxidation of milk and milk products: a review. Crit Rev Food Sci Nutr 7:13–37
Sayre LM, Zelasko DA, Harris PLR, Perry G, Salomon RG, Smith MA (1997) 4-hydroxynonenal-derived advanced lipid peroxidation end products are increased in Alzheimer's disease. J Neurochem 68:2092–2097
Schaich KM, Karel M (1976) Free radical reactions of peroxidizing lipids with amino acids and proteins: an ESR study. Lipids 11:392–400
Schebor C, Buera MP, Karel M, Chirife J (1999) Color formation due to non-enzymatic browning in amorphous, glassy, anhydrous, model systems. Food Chem 65:427–432
Sell DR, Monnier VM (1989) Structure elucidation of a senescence cross-link from human extracellular matrix. J Biol Chem 264:21597–21602
Shacter E (2000) Quantification and significance of protein oxidation in biological samples. Drug Metab Rev 32:307–326
Shahidi F, Amarowicz R (1996) Antioxidant activity of protein hydrolyzates from aquatic species. J Am Oil Chem Soc 73:1197–1199
Shahidi F, Han XQ (1993) Encapsulation of food ingredients. Crit Rev Food Sci Nutr 33:501–547
Sherwin ER (1978) Oxidation and antioxidants in fat and oil processing. J Am Oil Chem Soc 55:809–814
Shimada Y, Roos Y, Karel M (1991) Oxidation of methyl linoleate encapsulated in amorphous lactose-based food model. J Agric Food Chem 39:637–641
Shinmoto H, Dosako S, Nakajima I (1992) Anti-oxidant activity of bovine lactoferrin on iron/ascorbate induced lipid peroxidation. Biosci Biotech Biochem 12:2079–2080
Sims RJ, Fioriti JA, Trumbetas J (1979) Effect of sugars and sugar alcohols on autoxidation of safflower oil in emulsions. J Am Oil Chem Soc 56:742–745
Singh R, Barden A, Mori T, Beilin L (2001) Advanced glycation end-products: a review. Diabetologia 44:129–146
Slade L, Levine H, Reid DS (1991) Beyond water activity: recent advances based on an alternative approach to the assessment of food quality and safety. Crit Rev Food Sci Nutr 30:115–360

Smith CD, Carney JM, Starke-Reed PE, Oliver CN, Stadtman ER, Floyd RA, Markesbery WR (1991) Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. Proc Natl Acad Sci U S A 88:10540–10543
Smith MA, Taneda S, Richey PL, Miyata S, Yan S-D, Stern D, Sayre LM, Monnier VM, Perry G (1994) Advanced Maillard reaction end products are associated with Alzheimer disease pathology. Proc Natl Acad Sci U S A 91:5710–5714
Spiteller G (2005) The relation of lipid peroxidation processes with atherogenesis: a new theory on atherogenesis. Mol Nutr Res 49:999–1013
Stadler RH, Blank I, Varga N, Robert F, Hau J, Guy PA, Robert M-C, Riediker S (2002) Food chemistry: acrylamide from Maillard reaction products. Nature 419:449–450
Stadtman ER (1992) Protein oxidation and aging. Science 257:1220–1224
Stadtman ER (2001) Protein oxidation in aging and age-related diseases. Ann NY Acad Sci 928:22–38
Stadtman ER, Berlett BS (1998) Reactive oxygen-mediated protein oxidation in aging and disease. Drug Metab Rev 30:225–243
Stadtman ER, Levine RL (2000) Protein oxidation. Ann NY Acad Sci 899:191–208
Stadtman ER, Levine RL (2003) Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino Acids 25:207–218
Stadtman ER, Starke-Reed PE, Oliver CN, Carney JM, Floyd RA (1992) Protein modification in aging. EXS 62:64–72
Stapelfeldt H, Nielsen BoR, Skibsted LH (1997) Effect of heat treatment, water activity and storage temperature on the oxidative stability of whole milk powder. Int Dairy J 7:331–339
Starke-Reed PE, Oliver CN (1989) Protein oxidation and proteolysis during aging and oxidative stress. Arch Biochem Biophys 275:559–567
Stringer MD, Görög PG, Freeman A, Kakkar VV (1989) Lipid peroxides and atherosclerosis. Br Med J 298:281–284
Suetsuna K, Ukeda H, Ochi H (2000) Isolation and characterization of free radical scavenging activities peptides derived from casein. J Nutr Biochem 11:128–131
Sun Q, Senecal A, Chinachoti P, Faustman C (2002) Effect of water activity on lipid oxidation and protein solubility in freeze-dried beef during storage. J Food Sci 67:2512–2516
Tenada S, Monnier VM (1994) ELISA of pentosidine, an advanced glycation end product, in biological specimens. Clin Chem 40:1766–1773
Tewa-Tagne P, Briançon S, Fessi H (2007) Preparation of redispersible dry nanocapsules by means of spray-drying: development and characterisation. Eur J Pharm Sci 30:124–135
Thornalley PJ, Langborg A, Minhas HS (1999) Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. Biochem J 344:109–116
Uchida K, Stadtman ER (1992) Modification of histidine residues in proteins by reaction with 4-hydroxynonenal. Proc Natl Acad Sci U S A 89:4544–4548
Uchida K, Kato Y, Kawakishi S (1990) A novel mechanism for oxidative cleavage of prolyl peptides induced by the hydroxyl radical. Biochem Biophys Res Commun 169:265–271
Updike AA, Schwartz SJ (2003) Thermal processing of vegetables increases cis isomers of lutein and zeaxanthin. J Agric Food Chem 51:6184–6190
Uribarri J, Woodruff S, Goodman S, Cai W, Chen X, Pyzik R, Yong A, Striker GE, Vlassara H (2010) Advanced glycation end products in foods and a practical guide to their reduction in the diet. J Am Diet Assoc 110:911–916
van Het Hof KH, West CE, Weststrate JA, Hautvast JG (2000) Dietary factors that affect the bioavailability of carotenoids. J Nutr 130:503–506
Venolia AW, Tappel AL (1958) Brown-colored oxypolymers of unsaturated fats. J Am Oil Chem Soc 35:135–138
Voziyan PA, Hudson BG (2005) Pyridoxamine: the many virtues of a Maillard reaction inhibitor. Ann NY Acad Sci 1043:807–816
Wang H, Qian H, Yao W (2011) Melanoidins produced by the Maillard reaction: structure and biological activity. Food Chem 128:573–584

Watanabe K, Sato Y, Kato Y (1980) Chemical and conformational changes of ovalbumin due to the Maillard reaction. J Food Process Preserv 3:263–274

Wells-Knecht KJ, Zyzak DV, Litchfield JE, Thorpe SR, Baynes JW (1995) Mechanism of autoxidative glycosylation: identification of glyoxal and arabinose as intermediates in the autoxidative modification of proteins by glucose. Biochemistry 34:3702–3709

Wilhelm E, Battino R, Wilcock RJ (1977) Low-pressure solubility of gases in liquid water. Chem Rev 77:219–262

Williams AW, Boileau TWM, Erdman JW (1998) Factors influencing the uptake and absorption of carotenoids. Exp Biol Med 218:106–108

Wishner LA (1964) Light-induced oxidations in milk. J Dairy Sci 47:216–221

Wolfrom ML, Kashimura N, Horton D (1974) Factors affecting the Maillard browning reaction between sugars and amino acids. Studies on the nonenzymic browning of dehydrated orange juice. J Agric Food Chem 22:796–800

Wu W, Wu X, Hua Y (2010) Structural modification of soy protein by the lipid peroxidation product acrolein. Lebensm Wiss Technol 43:133–140

Yagi K (1987) Lipid peroxides and human diseases. Chem Phys Lipids 45:337–351

Yeboah FK, Alli I, Yaylayan VA (1999) Reactivities of D-glucose and D-fructose during glycation of bovine serum albumin. J Agric Food Chem 47:3164–3172

Yen G-C, Hsieh P-P (1995) Antioxidant activity and scavenging effects on active oxygen of xylose-lysine Maillard reaction products. J Sci Food Agric 67:415–420

Yilmaz Y, Toledo R (2005) Antioxidant activity of water-soluble Maillard reaction products. Food Chem 93:273–278

Zamora R, Hidalgo FJ (2005) Coordinate contribution of lipid oxidation and Maillard reaction to the nonenzymatic food browning. Crit Rev Food Sci Nutr 45:49–59

Zhou P, Guo M, Liu D, Liu X, Labuza TP (2013) Maillard-reaction-induced modification and aggregation of proteins and hardening of texture in protein bar model systems. J Food Sci 78:437–444

Zirlin A, Karel M (1969) Oxidation effects in a freeze-dried gelatin-methyl linoleate system. J Food Sci 34:160–165

Chapter 15
Improvement of Bioaccessibility and Bioavailability: From Molecular Interactions to Delivery Systems

Maarit J. Rein and Marcia da Silva Pinto

Abstract Although there are many studies reporting the potential health benefits of the diverse bioactive compounds present in our diet, many aspects related to their bioavailability and bioaccessibility, and particularly their metabolism, have been poorly investigated. In order to exert an effect in the body, a compound should be able to reach the target site of action at concentrations that will trigger the expected response. When considering the potential beneficial effects of bioactive food compounds, it is important to take into account the many factors that can have a significant effect on their bioavailability, and consequently can alter the amount of these compounds that will be absorbed and that will ultimately reach the site of action. This chapter focuses on the several means of improving bioavailability that are described in the literature, including bioaccessibility, food matrix, biological transporters, metabolizing enzymes, and molecular interactions.

Keywords Bioavailability · Bioaccessibility · Bioefficacy · Food · Bioactive compounds · Processing · Nanosystems · Molecular interactions

15.1 Introduction

Food is our fuel. In earlier times, when the gain of nutrients such as vitamins and minerals was much lower, food had a different meaning and function than it does today. Today food is for enjoyment as well as nourishment, and when consuming food, we aim to benefit from bioactive food compounds other than just the essential nutrients that keep our engines running. Nowadays, nutritional research is focused on health promotion, disease prevention, performance improvement, and risk assessment (Kussmann et al. 2007). When we discuss the health-promoting or disease-preventive properties of food, we aim for long-term physiological effects arising from many different bioactive food compounds.

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The bioactive food compounds responsible for the positive effects on our well-being are derived mostly from the plant kingdom, but some originate also from animal sources, such as those from dairy products. Multiple epidemiological studies over the course of many years have shown that diets rich in vegetables and fruits promote health and reduce the risk of certain chronic diseases. Through these studies it has been determined that five portions of fruits or vegetables a day is good for our health. Fruits and vegetables contain many bioactive compounds which are secondary metabolites in plants and are considered potential compounds for maintaining human health (Patil et al. 2009). These include both lipophilic compounds such as carotenoids, and hydrophilic compounds such as phenolic acids.

It is a complex task to demonstrate a health benefit of such a vast range of different compounds as the bioactive food compounds can be. Several different assessment steps are needed for a given compound to prove a health benefit. The compound of interest must first be released from the food matrix and become bioaccessible, and must then go through metabolism, and finally reach the target tissue where it can exert its beneficial action on health. The assessments of bioaccessibility and bioavailability of health-associated compounds are critical stages of food nutrition research toward understanding the potentials of these food compounds.

Bioaccessibility is defined as the proportion of a compound which is released from the food matrix during digestion in the mouth and gastrointestinal tract, and hence becomes available for absorption through the gut wall into the bloodstream. Bioavailability is defined as the proportion of a given food that the body can actually take up and use, being a matter of nutritional efficacy (Benito and Miller 1998). Bioavailability addresses several processes including liberation from the matrix, absorption, distribution, metabolism, and elimination phases (LADME). We as food scientists often focus on the end product and its benefits with the bioactive compounds, and overlook the LADME factors that affect the aimed benefit, whether nutritional or functional. For the bioactivity expectation, it is important to note that only a proportion of a nutrient or a bioactive compound from food will be effectively used by an organism (Fernandez-Garcia et al. 2009).

Bioavailability of nutrients is studied through human intervention studies looking at the nutrient pharmacokinetics with area under the curve (AUC), maximum concentration (C_{max}), and time of maximum concentration (t_{max}) measurements. AUC corresponds to the fraction of the ingested nutrient that reaches the systemic circulation. It is therefore measured from plasma samples as a function of time and is expressed in mg*h/L. The area under the curve is dependent on the amount of the ingested food, i.e. digested nutrient, and the rate of elimination of the studied nutrient from the body. In general, the concentration of a nutrient in the blood increases linearly with increasing dose. However, for many nutrients there is a threshold of absorption above which their concentration in the blood reaches a plateau. C_{max} is the measurement of the highest concentration of an investigated nutrient in the blood. The time at which C_{max} is observed is referred to as t_{max}.

Many factors influence the bioavailability of nutrients, and they can be broadly divided into external factors, product-related factors, and physiological factors. A major external factor known to affect the bioavailability of an ingredient is the

target group. The level of absorption of many compounds varies among age groups, for example, due to the status and composition of the intestinal microbiota and the release of bile. Consumption habits, another example of an external factor, also affect the bioavailability of nutrients. A clear demonstration of this is evident when lipid-soluble nutrients like vitamins A, D, E, and K, and carotenoids are better absorbed when consumed with other dietary lipids.

A product-related factor often found to affect the bioavailability of nutrients can be linked to the amount of a nutrient in a product and the molecular structure of the particular nutrient, i.e. the physical form (solid *vs.* liquid), chemical form (crystalline *vs.* salt), and whether the molecule is coupled to another nutrient or a protein. These factors can significantly affect the solubility and stability of the nutrient, and therefore its bioavailability. Furthermore, the chemical structure of the nutrient might also affect the site of absorption in the gut, which is linked to physiological factors affecting bioavailability—for example, whether a nutrient is absorbed by passive or active transport into the systemic circulation. In some cases, the stereo configuration of the nutrient similarly affects the distribution of the compound in the body, because some transport proteins have a higher affinity to one stereoisomer form than the other. Physiological factors are also important in terms of bioavailability. For example, some individuals are described as being either high or low absorbers of a specific compound. This has been linked to the composition and activity of the intestinal microbiota, but also to the presence and activity of the different digestive enzymes, the ability to emulsify lipophilic nutrients, and the activity of the transport systems.

The limited bioavailability of bioactive compounds hinders their use as functional ingredients. It is only by understanding the mechanisms of the absorption of bioactive compounds, the possibilities of different delivery systems that can be used, and the ways to protect these molecules during the whole chain from ingredient manufacturing to product formulation and storage conditions, that the bioavailability of bioactive compounds can be enhanced and their efficiency improved.

15.2 Improvement of Bioaccessibility and Bioavailability

Food bioaccessibility has not been investigated to a great extent, and it seems that scientists up to now have put more effort into studying and understanding nutrient bioavailability than bioaccessibility (Patil et al. 2009). This is understandable from the perspective that bioavailability is the limiting step in bioefficacy. However, that is not the case if the bioactive compounds are not liberated from the food matrix in the first place. Aiding the bioaccessibility of bioactive compounds from foods through different means of delivery systems, processing, and molecular modifications can promote both the bioavailability and bioefficacy of bioactive compounds (Fig. 15.1).

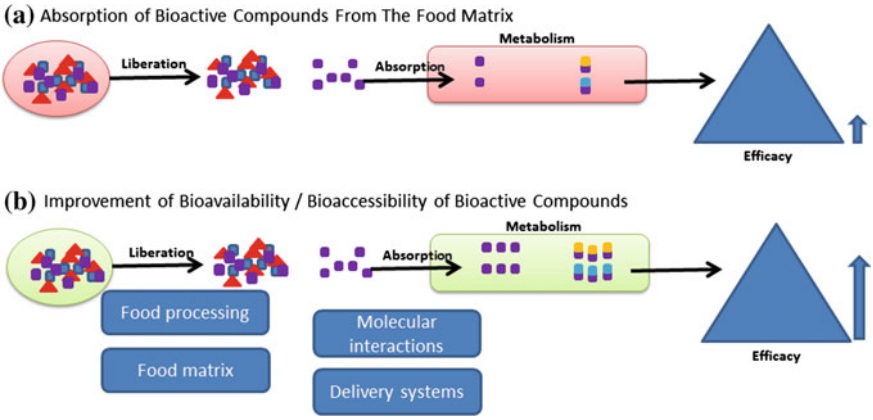

Fig. 15.1 Basic steps involved in the bioavailability/bioaccessibility of bioactive food compounds. **a** Regular absorption of bioactive compounds from the food matrix that generally results in lower efficacy. **b** Methods/techniques for improving absorption of food compounds aiming to increase efficacy

15.2.1 Food Processing

Food processing takes many different forms, from mixing to milling, drying to cooking, fermenting to roasting, and so forth. Various processing steps can affect the bioactive food compounds, whether of plant or animal origin, either destructively or beneficially.

Processing of plant foods can influence the bioaccessibility of digestible starch, liposoluble vitamins and carotenoids, and other nutrients. For example, changes in cell wall structure and properties or isomerization and other structural changes in bioactive molecules can occur due to processing steps such as heating (Colle et al. 2013). These changes can then alter the bioaccessibility and bioavailability of the different food compounds, carotenoids having been studied extensively in this respect. It has been shown that cooking and pureeing carrots improves in vitro bioaccessibility and bioavailability of carotenoids (Netzel et al. 2011), and significantly more β-carotene is absorbed by human ileostomy patients from meals containing cooked, pureed carrots than from meals containing the raw vegetable (Livny et al. 2003). Processing raw tomatoes into tomato paste has been shown to increase the bioavailability of lycopene, a major carotenoid in these vegetables. The addition of lipids prior to processing of tomatoes also enhances lycopene bioaccessibility (Colle et al. 2013); however, it seems that the effect of fat is selective for the type of carotenoid and appears to be dependent on the food matrix (Victoria-Campos et al. 2013). Homogenization of wolfberry in hot skimmed milk was found to significantly improve the bioavailability of zeaxanthin, another carotenoid pigment in plants, in healthy humans when compared to conventional homogenization with hot water or with warm milk. The presence of milk proteins and high temperature improved

zeaxanthin bioavailability through incorporation into mixed micelles, uptake to enterocytes, and release in lipoproteins, although the exact mechanism still remains unclear (Benzie et al. 2006). A majority of studies relating to processing effects on carotenoids have demonstrated a positive effect of cooking on bioaccessibility and bioavailability. In the case of red peppers, however, the opposite was observed. Different carotenoid pigments were more bioaccessible in in vitro experiments when digested raw, and grilling and boiling reduced the bioaccessibility of most free and esterified pigments of green and red peppers (Victoria-Campos et al. 2013). The same observation was made with fruit juice beverages containing milk, where high-pressure temperature and thermal treatment did not facilitate carotenoid bioaccessibility. It was thus concluded that higher amounts of total carotenoids in beverages as a consequence of processing do not ensure an increase in bioaccessibility. The controversial bioaccessibility results found with carotenoids from different plant origins indicate that the effects of food processing on bioactive food compound bioaccessibility and bioavailability are much more complex than the largely positive effects expected in relation to mechanical or thermal processing involving plant cell wall disruption (Cilla et al. 2012).

15.2.2 Matrix Effect

Interactions between different food components influence the bioaccessibility and bioavailability of the digested food (Fernández-García et al. 2009; Shen et al. 2011). The physicochemical properties of the food and the surrounding environment in the digestive system such as pH, concentrations, and intermolecular forces affect the bioaccessibility and bioavailability of the food compounds (Neilson and Ferruzzi 2011; Serra et al. 2013). In a simulated gastrointestinal digestion of cooked seafood products, the bioaccessibility of fluoride was increased by a lower pH, the presence of cations, and a higher concentration of bile salts (Rocha et al. 2013).

There is increasing evidence that dietary fibers play an important role in the bioaccessibility of nutrients and bioactive compounds during digestion. In a simulated digestion, delivery of microencapsulated tuna oil from a cereal bar was hindered in comparison to that from orange juice or yogurt, most likely due to the high content of fat and dietary fiber in the matrix (Shen et al. 2011). In studies comparing the digestion of bioactive compounds from vegetables such as broccoli or cabbage with the digestion of pure bioactive compounds, the vegetable matrix improved the bioavailability in an in vitro model with the latter and in a rat model with the former vegetable (Keck et al. 2003; Lee et al. 2014). On the other hand, plant cell walls are largely resistant to degradation in the upper gut, representing an important barrier for the release of bioactive compounds. One such example is ferulic acid, a phenolic acid in whole grains, which has limited bioavailability due to its high binding to polysaccharides, i.e. dietary fibers. Mateo Anson et al. (2009) studied the bioaccessibility of ferulic acid from wheat fractions and baked breads. The authors observed that wheat ferulic acid had low bioaccessibility (<1 %), but

that bioaccessibility was high when free ferulic acid was added to baking flour (~60 %), because it was not bound to polysaccharides. Similarly, when cocoa cream was enriched with proanthocyanidins, a condensed tannin type of polyphenol, and fed to rats, the metabolite profile of rat plasma was different from the plasma metabolite profile of non-enriched food given to rats, indicating that formulation or fortification of bioactive compounds was a potential means of improving bioavailability (Serra et al. 2013).

Improved bioavailability through improved bioaccessibility can be seen in the following example of phytosterols, which are plant sterols with a chemical structure similar to that of cholesterol. When phytosterols were added to food products, changes in product texture appeared due to recrystallization. The crystalline phytosterols cannot be absorbed in the intestine, thus resulting in very low bioaccessibility; however, synthesized colloidal phytosterols have better solubility, which improved their bioavailability (Rossi et al. 2010). More recently, crystalline deposition of bioactive molecules was studied and the bioavailability of β-carotene and lycopene from carrots, tomatoes, and papaya were compared in a randomized crossover trial with healthy subjects. The bioavailability of β-carotene from papaya was found to be ca. three times as high as that from carrots and tomatoes. The possible explanation is related to the liquid crystalline deposition of β-carotene, and the storage of lycopene in very small crystalloids in papayas was found to be associated with their high bioavailability (Schweiggert et al. 2013).

It has been recognized that foods with high caloric and fat content promote improved bioavailability through their effects on intestinal physiology (Mullen et al. 2008a, b; Welch et al. 1988a, b). For example, Walsh et al. (2003) proposed through an in vitro stimulation that the bioavailability of isoflavonoids from foods containing fat and protein exceeded that of isoflavonoid supplements consumed without food. In a study using a sophisticated gastrointestinal model (TIM-1 model) investigating the effects of a high-fat meal matrix and protein complexation on blueberry anthocyanin bioaccessibility, it was shown that blueberry-enriched defatted soybean flour anthocyanin samples were protected during the upper gut transit by the co-existing proteins, resulting in higher recovery from the TIM-1 system compared to plain blueberry juice anthocyanin samples (Ribnicky et al. 2014). The addition of olive oil as an oil-in-water emulsion during digestion has also been demonstrated to increase carotenoid bioaccessibility in their micellar uptake from tomato- and carrot-derived purées (Moelants et al. 2012). Fat also increased the bioaccessibility of chlorophyll in a digestive model (Victoria-Campos et al. 2013).

Few scientific publications have focused on the effect of the food matrix in modulating the bioavailability of coffee phenolics and flavonoids present in cocoa and tea products. Some studies have looked at the effect of milk proteins on modulating the bioavailability of coffee chlorogenic acids. Using in vitro/ex vivo modeling, Dupas et al. (2006) showed that 40 % of added 5-caffeoylquinic acid binds to casein. Renouf et al. (2010) studied the effect of adding milk or sugar/non-dairy creamer to coffee on the bioavailability of phenolic acid equivalents in humans. When 10 % whole milk was added to coffee, no significant difference was observed in AUC, C_{max}, or t_{max} of caffeic acid, ferulic acid, or isoferulic acid equivalents

compared to coffee alone. However, when sugar/non-dairy creamer was used, some differences in C_{max} (lower) and t_{max} (longer) were observed compared to plain coffee. The overall delivery of chlorogenic acid metabolites was not significantly different between treatments, but the addition of sugar/non-dairy creamer to coffee led to significant changes in the plasma appearance of those metabolites.

The food matrix does not influence the bioaccessibility and bioavailability of bioactive compounds in all cases, as was shown with hesperidin, a citrus fruit bioactive belonging to the flavanone subgroup of flavonoids. Consumption of orange juice with full-fat yogurt did not significantly affect the C_{max} or t_{max} values of hesperidin (Mullen et al. 2008a, b). Food processing also had no effect on hesperidin bioaccessibility and bioavailability when comparing the consumption of orange fruits with the consumption of orange juice (Brett et al. 2009). Controversial results have been reported with regard to the effect of milk protein on the bioaccessibility of epicatechin, the flavan-3-ol subgroup of polyphenols present in significant quantities in foods such as cocoa, teas, and apples (Rein et al. 2013). Serafini et al. (2003) reported an unfavorable effect of milk on the bioaccessibility of epicatechin, showing a lower AUC when chocolate was ingested with milk. However, several other studies have demonstrated the nonexistence of such an effect of milk (Schroeter et al. 2003; Keogh et al. 2007; Roura et al. 2007, 2008). It was recently proposed that milk reduces the urinary excretion but not plasma pharmacokinetics of cocoa flavan-3-ol metabolites in humans (Mullen et al. 2009). In addition to the effect of milk, Neilson et al. (2009) concluded that the physical form of the food and the sucrose content may influence the t_{max} and C_{max} of cocoa flavan-3-ols. With regard to tea, in general, it is suggested that tea with milk has a negligible effect on the bioavailability and bioaccessibility of the flavan-3-ols (Reddy et al. 2005; Van der Burg-Koorevaar et al. 2011).

When addressing the bioaccessibility and bioavailability of bioactive food compounds, it is necessary to evaluate not only the digested source of these nutrients, but the effect of the whole meal ingested at the same time. Such bioaccessibility data can be used to support the risk/benefit estimation of vitamin and mineral intake (Rocha et al. 2013) as well as that of other bioactive compounds.

15.2.3 Molecular Interactions

Factors influencing bioavailability include physical and chemical properties such as hydrophobicity, pK_a, and solubility. On the other hand, bioavailability has some connection with permeability, efflux transporters (e.g. P-glycoprotein, P-gp), and enzyme induction or inhibition on intestinal epithelial cells. The molecular structure of a compound has a considerable effect on its absorption and therefore its bioavailability (Scholz and Williamson 2007). Molecular weight is an important factor to consider; for example, high molecular weight compounds such as the oligomeric proanthocyanidins and complex lipids do not pass through the intestinal cells unless they are broken down (Deprez et al. 2000; Appeldoorn et al. 2009).

According to Leucuta (2014), the requirements for absorption of chemical compounds are solubility and permeability, both aspects highly influenced by lipophilicity. The same is applied to bioactive food compounds; McClements (2013) reported that the bioavailability of many lipophilic bioactive compounds was relatively low due to their poor solubility, permeability, and/or chemical stability within the human gastrointestinal tract. In a double-blind, randomized, placebo-controlled crossover study using healthy volunteers, Ramprasath et al. (2013) reported that due to structural differences between krill oil, a phospholipid-based oil, and fish oil, a triglyceride-based oil, the bioavailability of krill oil was higher than that of fish oil, resulting in a more effective increase of *n*-3 polyunsaturated fatty acid (PUFA) levels. Cruz-Hernandez et al. (2012) reported improved bioavailability of eicosapentaenoic acid (EPA) when delivered by structured and free monoacylglycerols (MAG) in a preclinical model of lipid malabsorption. Moran et al. (2013) studied the metabolic differences in an animal model between lycopene, the major tomato carotenoid, and phytoene, a minor tomato carotenoid. Although phytoene is a minor component in tomatoes, it is found in human blood and tissues in concentrations similar to those of lycopene. The two carotenoids differ not only structurally by saturation, but also in terms of bioavailability, tissue deposition, and clearance. The results of this study suggest that phytoene has greater bioavailability and is cleared more slowly than lycopene.

Some bioactive food compounds are attached to sugar moieties such as β-glucosides that can be absorbed and metabolized by enzymes (e.g. β-glucosidases and lactase-phlorizin hydrolase [LPH]) in the small intestine (Hollman et al. 1999). However, when the compounds are attached to an additional rhamnose moiety, they need to reach the large intestine to have the sugar moieties cleaved off by the intestinal microbiota before absorption (Erlund et al. 2000). Nielsen et al. (2006a, b) studied the improvement in the bioavailability of hesperidin by enzymatic modification in a randomized double-blind crossover trial in humans. Hesperidin was reported as having low bioavailability due to its rutinoside moiety. The authors noted that the consumption of orange juice treated with hesperidinase, leading to the conversion of hesperidin to hesperetin-7-glucoside, significantly and favorably affected bioavailability parameters such as AUC, C_{max}, and t_{max}.

Apart from the chemical structure of food bioactive compounds, their isomeric configuration can also affect their absorption. This is the case for (−)-epicatechin and (+)-catechin bioavailability (Ottavianni et al. 2011) as well as *cis*-isomers and all-*trans* isomer of lycopene bioavailability (Boileau et al. 2002), for the biological activity of (R-, S-) equol (Muthyala et al. 2004), and for the metabolism of (R-, S-) hesperidin (Lévèques et al. 2012). Jensen et al. (2006) studied the bioavailability of α-tocopherol stereoisomers in a rat model fed for 10 days a diet containing either α-tocopherol—also referred to as *RRR*-α-tocopherol (2,5,7,8-tetramethyl-2R-(4'R,8'R,12-trimethyltridecyl)-6-chromanol)), and which has the highest reported biological activity—or all-*rac*-α-tocopheryl acetate (2,5,7,8-tetramethyl-2RS-(4'RS,8'RS,12-trimethyltridecyl)-6-chromanol), a mixture of isomers of synthetic origin and widely used to supplement food. The authors observed that increasing dietary levels of all-*rac*-α-tocopheryl acetate led to a decrease in the proportion of

RRR-α-tocopherol found in plasma, whereas the other stereoisomers were not affected. This indicated that in a racemic mixture, the presence of other stereoisomers hindered the absorption of *RRR*-α-tocopherol.

Other important factors affecting the bioavailability of bioactive food compounds are the different transport mechanisms taking place in the intestinal lumen, including passive diffusion, facilitated diffusion, and active transport (Rein et al. 2013). Therefore, potential ways of improving the bioavailability of bioactive food compounds could emerge through the competition and inhibition of intestinal cell transporters (Scheepens et al. 2010). The ATP-binding cassette (ABC) family of transporters, including the intestinal P-gp (P-glycoprotein) efflux pump, have been reported as a major contributor to the low bioavailability of a number of compounds (Xie et al. 2011). For example, in vitro experiments suggest that the bioavailability of the flavonoid hesperidin may be enhanced by inhibiting the ABC transporters by competitive exposure to other flavonoids such as quercetin, resulting in a decrease in the efflux of hesperidin (Brand et al. 2008). The same approach has been suggested for (−)-epigallocatechin 3-gallate (EGCG), where the combination of the bioactive with naturally occurring inhibitors of efflux proteins resulted in increased cytosolic levels of the compound (Hong et al. 2003).

Membrane transporters are involved in two mechanisms related to the permeability of compounds: uptake and efflux. Vitamin transporters, the glucose transporter (GLUT) family, sodium-glucose linked transporter (SGLT) family, and organic anion transporter 1 (OAT1), amongst others, are involved in the uptake of compounds, enhancing their transport across the intestine (Kerns and Di 2008). Whitley et al. (2005) studied the ability of organic anion transporters (OATs) and organic anion-transporting polypeptides (OATPs) to transport ellagic acid, a phenolic acid found in berries and nuts. OATs play a critical role in the distribution and elimination of a diverse array of exogenous and endogenous compounds. The authors reported that the interaction between hOAT1 and rOat1 with ellagic acid was of high affinity, suggesting the potential combination of ellagic acid with other compounds in order to improve their bioavailability.

Cytochrome (CYP) enzymes are responsible for the breakdown of endogenous and exogenous compounds into metabolites, with various CYP forms present in the human liver. Flavonoids have been identified as inhibitors of cytochrome P450 enzymes (Dresser and Bailey 2003). Kimura et al. (2010) studied the inhibitory effects of polyphenols on human cytochrome P450 3A4 and 2C9 activity by in vitro assays. The results showed that three coumarins and 12 flavonoids significantly suppressed CYP3A4 or CYP2C9 activity, and among these compounds, galangin, chrysin, and apigenin, which are present in propolis, had the most potent inhibitory effect against these CYP isoforms. These findings suggest that dietary compounds, and particularly flavonoids, may have the potential to inhibit the metabolism of other compounds such as clinical drugs.

As bioactive food compounds are present in our diet in many different forms, understanding and elucidating their chemical properties is crucial when studying the bioavailability and potential bioefficacy of these compounds with regard to human health.

15.2.4 Delivery Systems

The use of nanoparticles has been reported as a promising means of improving the delivery of compounds with low bioavailability, leading to enhanced efficacy (Li and Huang 2008). However, the main emphasis in the design of nanosystems is improving local bioavailability. For example, nanotechnology is used to increase compound delivery to the diseased target tissue (e.g. vessel wall, tumor, or brain) in addition to improving the therapeutic index or avoiding toxicity (Yliperttula and Urtti 2008).

In principle, the use of nanosystems could facilitate the transport of the compound across biological barriers, as well as avoiding the metabolic modifications that could lead to low absorption. However, it is very important to understand from an efficacy perspective that the choice of carrier material in the oral delivery system is extremely important, because it significantly affects the pharmacokinetics and pharmacodynamics of the ingested compounds (EFSA Guidelines 2011; Xie et al. 2011). Xie et al. (2011) developed PLGA [poly (lactic-co-glycolic acid)] nanoparticles to improve the bioavailability of curcumin, a low molecular weight hydrophobic polyphenol that is extracted from turmeric, using a rat model. The bioavailability of curcuminoids is low due mainly to their poor water solubility and rapid metabolism. After oral administration of curcumin nanoparticles, relative bioavailability was 5.6-fold higher and had a longer half-life compared with the native curcumin. Neves et al. (2013) reported the use of lipid nanoparticles for improving the bioavailability of resveratrol, a polyphenol found in grapes and red wines. This compound has been described as having poor bioavailability and low water solubility, and as being chemically unstable. The authors conducted in vitro release studies on conditions of storage, and concluded that the lipid nanoparticles could be considered suitable carriers for oral administration, conferring protection to the incorporated resveratrol and allowing a controlled release after uptake. Tzeng et al. (2011) reported the use of a nanoparticle engineering process to enhance the dissolution and antioxidant activity of kaempferol, a flavonoid widespread in food sources but with limited clinical application due to poor dissolution properties. The authors suggested that kaempferol nanoparticles could be considered a low-dose alternative to kaempferol in health food and future clinical research.

The application of either encapsulation or emulsions is also an effective strategy for achieving several objectives, including improved bioaccessibility and stability of the bioactive compounds. For example, Yu et al. (2012) developed a food-grade curcuminoid organogel with high bioaccessibility and high loading of curcumin. Organogel-based delivery systems are relatively new in food science. Organogels are formed by liquid oils trapped by the extensive crystalline networks of organogelators, such as monoglycerides, fatty acids, and fatty alcohols. In another study, Rossi et al. (2010) reported the synthesis of colloidal phytosterols and evaluated their bioaccessibility in an in vitro model that resulted in effective solubilization in model dietary mixed micelles, and the micellar cholesterol concentration was effectively reduced by almost 50 % within 2 h. Fernandez-Garcia et al. (2008) developed an emulsifier system to improve the bioaccessibility of carotenoids.

Bioaccessibility of these compounds from natural sources (mainly fruits and vegetables) is often low and is conditioned by different factors, mainly the processing state of the food and the matrix composition. One of the key factors affecting bioaccessibility is the amount and type of fat present in the food. A minimum amount of fat is required for increasing absorption, so formulation of carotenoids in an oily matrix (vegetable oils) may provide high bioaccessibility. However, as was previously discussed in this chapter, it has been shown that the bioaccessibility of carotenoids from fatty food formulations is not as high as expected. Walde et al. (2013) compared the bioavailability of dietary tocotrienols from barley and palm oils in an animal model. The main component of tocotrienols from barley is α-tocotrienol, while palm oil tocotrienols are particularly rich in γ-tocotrienol. Nanoemulsification of barley oil led to higher tocotrienol levels than with non-emulsified oil, resulting in high proportions of α-tocotrienol. In addition, the authors observed that tocotrienol content in hens fed barley oil was significantly higher than in those fed palm oil, suggesting that α-tocotrienol is better absorbed than γ-tocotrienol.

Since the application of nanoparticles is still a new field in the nutrition and food science areas, it is important to emphasize that it must be evaluated in preclinical toxicological tests and in human clinical trials. Nonetheless, many authors have reported that safety problems are expected to be no greater with nano-delivered compounds than with regular compounds (Yliperttula and Urtti 2009).

15.3 Human Studies and Efficacy

Although numerous studies have reported the potential health benefits of the diverse bioactive food compounds, many aspects related to their bioavailability, and particularly their metabolism, have been poorly investigated. In recent years, there has been an increase in the number of human intervention studies in the nutrition area; however, until now the majority of studies on the bioefficacy of bioactive food compounds have typically been based on in vitro models evaluating the parent compound at concentrations far higher than the physiological range. Therefore, when considering their potential beneficial effects, there are still many aspects that need a better understanding, especially with regard to the main compounds and their metabolites responsible for the health benefits and mechanisms of action (da Silva Pinto 2013).

The fact is that, today, there is an even stronger need for well-controlled long-term human studies in order to fully comprehend the metabolic and potential health effects of bioactive food compounds. It is important to understand that when planning any study, the choice of experimental design and statistical methods of analysis are crucial, since this choice will be reflected in the outcome of the analysis and the conclusions drawn (Chow and Liu 2009). Another important point that researchers should consider when planning human intervention studies to evaluate potential health benefits associated with bioactive compounds is that not only must care be taken in the experimental design, but the hypothesis must be very well defined prior to the study, as well as the biological relevance in the context of overall diet.

Because most food sources contain a mixture of potential bioactive compounds, it is also important to highlight the current need to identify and investigate the bioefficacy of the circulating metabolites, since it is most likely these compounds, and not the parent compounds, that will reach the various sites of action. In addition, since individual subjects differ widely in their physiological and metabolic responses to the compounds, the knowledge of inter- and intra-subject variability provides valuable information for assessing bioavailability. In general, in order to improve the intra-subject variability in bioavailability comparisons, a crossover design is often considered.

Indeed, care should be taken not only to consider statistical significance as the aim of the intervention study, but also the biological relevance of the primary objective/outcome that must be clearly defined prior to the study. For example, a statistically significant difference in the comparison of bioavailability between compounds does not necessarily imply that there is a clinically significant difference between them, since they can still achieve the same therapeutic effect (Chow and Liu 2009).

15.4 Conclusions and Future Perspectives

Bioaccessibility and bioavailability are crucial steps in assessing the potential health benefits of bioactive food compounds, and evaluating these steps is important in understanding the relationship between food and nutrition. Bioavailability addresses several phases, including liberation from the matrix, absorption, distribution, metabolism, and elimination (LADME). Scientists up to now have put more effort into studying nutrient bioavailability than bioaccessibility, although aiding the bioaccessibility of nutrients and bioactive compounds from foods through different processing techniques, delivery systems, and molecular modifications are excellent means of promoting bioavailability, and consequently bioefficacy. The various methods of improving bioaccessibility and bioavailability are all aimed at enabling the bioactive compound to exert a health benefit, improving performance, or managing risk.

Food processing can enhance the bioaccessibility of different compounds, for example, through disruption of plant cell walls, inducing molecular isomerization and other structural changes, influencing solubility, and improving micellar uptake of lipophilic bioactive compounds. Similar effects take place through changes in molecular interactions, which can influence the absorption of bioactive compounds through different transport mechanisms or impact metabolism and enzyme activity. The food matrix also plays an important role in bioaccessibility and bioavailability. The content of fat and dietary fiber in a given food can have a matrix effect, either hindering or improving nutrient bioavailability. Therefore, understanding the bioaccessibility and bioavailability of bioactive food compounds and the complexity of these processes is a key aspect in the formulation of functional foods.

The available data from bioaccessibility and bioavailability clinical studies can support the benefit and risk estimations of nutrients and other bioactive compounds. Although nanosystems have been investigated recently in terms of improving the bioavailability of clinical drugs, for nutrition we are still in the first stages of in vitro

and preclinical investigations. As food sources are a mixture of bioactive compounds, well-designed, long-term human studies are needed in order to gain an understanding of the potential health effects of these bioactive compounds in the context of overall diet. Human studies are also needed to validate the observations made in in vitro and preclinical studies on bioaccessibility and bioavailability before any claims of bioefficacy with bioactive food compounds can be considered.

References

Appeldoorn MM, Vincken J-P, Aura A-M, Hollman PCH, Gruppen H (2009) Procyanidin dimers are metabolized by human microbiota with 2-(3,4-dihydroxyphenyl)acetic acid and 5-(3,4-dihydroxyphenyl)-gamma-valerolactone as the major metabolites. J Agric Food Chem 57:1084–1092

Benito P, Miller D (1998) Iron absorption and bioavailability: An updated review. Nutrition Research 18:581–603

Benzie IFF, Chung WY, Wang J, Richelle M, Bucheli P (2006) Enhanced bioavailability of zeaxanthin in a milk-based formulation of wolfberry (Gou Qi Zi; *Fructus barbarum* L.). Br J Nutr 96:154–160

Boileau TW-M, Boileau AC, Erdman JW Jr (2002) Bioavailability of all-trans and cis-isomers of lycopene. Exp. Biol. Med. (Maywood) 227:914–919

Brand W, van der Wel PAI, Rein MJ, Barron D, Williamson G, van Bladeren PJ, Rietjens IMCM (2008) Metabolism and transport of the citrus flavonoid hesperetin in Caco-2 cell monolayers. Drug Metab Dispos 36:1794–1802

Brett GM, Hollands W, Needs PW, Teucher B, Dainty JR, Davis BD, Brodbelt JS, Kroon PA (2009) Absorption, metabolism and excretion of flavanones from single portions of orange fruit and juice and effects of anthropometric variables and contraceptive pill use on flavanone excretion. Br J Nutr 101:664–675

Chow S-C, Liu J-p (eds.) (2009) Introduction. In: Design and analysis of bioavailability and bioequivalence studies, 3rd edn. CRC, Chapman & Hall, p 3–11

Cilla A, Alegría A, de Ancos B, Sánchez-Moreno C, Cano MP, Plaza L, Clemente G, Lagarda MJ, Barberá R (2012) Bioaccessibility of tocopherols, carotenoids, and ascorbic acid from milk- and soy-based fruit beverages: influence of food matrix and processing. J Agric Food Chem 60:7282–7290

Colle IJP, Lemmens L, Van Buggenhout S, Met K, Van Loey AM, Hendrickx ME (2013) Processing tomato pulp in the presence of lipids: The impact on lycopene bioaccessibility. Food Res Int 51:32–38

Cruz-Hernandez C, Thakkar SK, Moulin J, Oliveira M, Masserey-Elmelegy I, Dionisi F, Destaillats F (2012) Benefits of structured and free monoacylglycerols to deliver eicosapentaenoic (EPA) in a model of lipid malabsorption. Nutrients 4:1781–1793

Da Silva Pinto M (2013) Tea: A new perspective on health benefits. Food Res Int 53:558–567

Déprez S, Brezillon C, Rabot S, Philippe C, Mila I, Lapierre C, Scalbert A (2000) Polymeric Proanthocyanidins Are Catabolized by Human Colonic Microflora into Low-Molecular-Weight Phenolic Acids. J Nutr 130:2733–2738

Dresser GK, Bailey DG (2003) The effects of fruit juices on drug disposition: a new model for drug interactions. Eur J Clin Invest 33(Suppl 2):10–16

Dupas CJ, Marsset-Baglieri AC, Ordonaud CS, Ducept FMG, Maillard M-N (2006) Coffee Antioxidant Properties: Effects of Milk Addition and Processing Conditions. J Food Sci 71: S253–S258

Erlund I, Kosonen T, Alfthan G, Mäenpää J, Perttunen K, Kenraali J, Parantainen J, Aro A (2000) Pharmacokinetics of quercetin from quercetin aglycone and rutin in healthy volunteers. Eur J Clin Pharmacol 56:545–553

European Food Safety Authority (EFSA). Scientific opinion: Guidence to the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain. 2011

Fernández-García E, Carvajal-Lérida I, Pérez-Gálvez A (2009) In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency. Nutr Res 29:751–760

Fernández-García E, Rincón F, Pérez-Gálvez A (2008) Developing an emulsifier system to improve the bioaccessibility of carotenoids. J Agric Food Chem 56:10384–10390

Hollman PC, Bijsman MN, van Gameren Y, Cnossen EP, de Vries JH, Katan MB (1999) The sugar moiety is a major determinant of the absorption of dietary flavonoid glycosides in man. Free Radic. Res. 31:569–573

Hong J, Lambert JD, Lee SH, Sinko PJ, Yang CS (2003) Involvement of multidrug resistance-associated proteins in regulating cellular levels of (−)-epigallocatechin-3-gallate and its methyl metabolites. Biochem. Biophys. Res. Commun. 310:222–227

Jensen SK, Nørgaard JV, Lauridsen C (2006) Bioavailability of alpha-tocopherol stereoisomers in rats depends on dietary doses of all-rac- or RRR-alpha-tocopheryl acetate. Br J Nutr 95:477–487

Keck A-S, Qiao Q, Jeffery EH (2003) Food matrix effects on bioactivity of broccoli-derived sulforaphane in liver and colon of F344 rats. J Agric Food Chem 51:3320–3327

Keogh JB, McInerney J, Clifton PM (2007) The effect of milk protein on the bioavailability of cocoa polyphenols. J Food Sci 72:S230–S233

Kerns EH, Di L (2008) Transporters. In: Drug-like properties: concepts, structure design and methods, from ADME to toxicity optimization, 1st edn. Elsevier, New York, pp 103–122

Kimura Y, Ito H, Ohnishi R, Hatano T (2010) Inhibitory effects of polyphenols on human cytochrome P450 3A4 and 2C9 activity. Food Chem Toxicol 48:429–435

Kussmann M, Affolter M, Nagy K, Holst B, Fay LB (2007) Mass spectrometry in nutrition: understanding dietary health effects at the molecular level. Mass Spectrom Rev 26:727–750

Lee H-R, Cho S-D, Lee WK, Kim G-H, Shim S-M (2014) Digestive recovery of sulfur-methyl-L-methionine and its bioaccessibility in Kimchi cabbages using a simulated in vitro digestion model system. J Sci Food Agric 94:109–112

Leucuta SE (2014) Selecting oral bioavailability enhancing formulations during drug discovery and development. Expert Opin Drug Discov 9(2) 139-150

Lévèques A, Actis-Goretta L, Rein MJ, Williamson G, Dionisi F, Giuffrida F (2012) UPLC-MS/MS quantification of total hesperetin and hesperetin enantiomers in biological matrices. J Pharm Biomed Anal 57:1–6

Li S-D, Huang L (2008) Pharmacokinetics and Biodistribution of Nanoparticles. Mol. Pharmaceutics 5:496–504

Livny O, Reifen R, Levy I, Madar Z, Faulks R, Southon S, Schwartz B (2003) Beta-carotene bioavailability from differently processed carrot meals in human ileostomy volunteers. Eur J Nutr 42:338–345

Mateo Anson N, van den Berg R, Havenaar R, Bast A, Haenen GRMM (2009) Bioavailability of ferulic acid is determined by its bioaccessibility. J Cereal Sci 49:296–300

McClements DJ (2013) Utilizing food effects to overcome challenges in delivery of lipophilic bioactives: structural design of medical and functional foods. Expert Opin Drug Deliv 10:1621–1632

Moelants KRN, Lemmens L, Vandebroeck M, Van Buggenhout S, Van Loey AM, Hendrickx ME (2012) Relation between particle size and carotenoid bioaccessibility in carrot- and tomato-derived suspensions. J Agric Food Chem 60:11995–12003

Moran NE, Clinton SK, Erdman JW Jr (2013) Differential bioavailability, clearance, and tissue distribution of the acyclic tomato carotenoids lycopene and phytoene in mongolian gerbils. J Nutr 143:1920–1926

Mullen W, Edwards CA, Serafini M, Crozier A (2008a) Bioavailability of pelargonidin-3-O-glucoside and its metabolites in humans following the ingestion of strawberries with and without cream. J Agric Food Chem 56:713–719

Mullen W, Archeveque M-A, Edwards CA, Matsumoto H, Crozier A (2008b) Bioavailability and metabolism of orange juice flavanones in humans: impact of a full-fat yogurt. J Agric Food Chem 56:11157–11164

Mullen W, Borges G, Donovan JL, Edwards CA, Serafini M, Lean MEJ, Crozier A (2009) Milk decreases urinary excretion but not plasma pharmacokinetics of cocoa flavan-3-ol metabolites in humans. Am J Clin Nutr 89:1784–1791

Muthyala RS, Ju YH, Sheng S, Williams LD, Doerge DR, Katzenellenbogen BS, Helferich WG, Katzenellenbogen JA (2004) Equol, a natural estrogenic metabolite from soy isoflavones: convenient preparation and resolution of R- and S-equols and their differing binding and biological activity through estrogen receptors alpha and beta. Bioorg. Med. Chem. 12:1559–1567

Neilson AP, Ferruzzi MG (2011) Influence of formulation and processing on absorption and metabolism of flavan-3-ols from tea and cocoa. Annu Rev Food Sci Technol 2:125–151

Neilson AP, George JC, Janle EM, Mattes RD, Rudolph R, Matusheski NV, Ferruzzi MG (2009) Influence of chocolate matrix composition on cocoa flavan-3-ol bioaccessibility in vitro and bioavailability in humans. J Agric Food Chem 57:9418–9426

Netzel M, Netzel G, Zabaras D, Lundin L, Day L, Addepalli R, Osborne SA, Seymour R (2011) Release and absorption of carotenes from processed carrots (Daucus carota) using in vitro digestion coupled with a Caco-2 cell trans-well culture model. Food Res Int 44:868–874

Neves AR, Lúcio M, Martins S, Lima JLC, Reis S (2013) Novel resveratrol nanodelivery systems based on lipid nanoparticles to enhance its oral bioavailability. Int J Nanomedicine 8:177–187

Nielsen ILF, Chee WSS, Poulsen L, Offord-Cavin E, Rasmussen SE, Frederiksen H, Enslen M, Barron D, Horcajada M-N, Williamson G (2006a) Bioavailability is improved by enzymatic modification of the citrus flavonoid hesperidin in humans: a randomized, double-blind, crossover trial. J Nutr 136:404–408

Nielsen ILF, Chee WSS, Poulsen L, Offord-Cavin E, Rasmussen SE, Frederiksen H, Enslen M, Barron D, Horcajada M-N, Williamson G (2006b) Bioavailability is improved by enzymatic modification of the citrus flavonoid hesperidin in humans: a randomized, double-blind, crossover trial. J Nutr 136:404–408

Ottaviani JI, Momma TY, Heiss C, Kwik-Uribe C, Schroeter H, Keen CL (2011) The stereochemical configuration of flavanols influences the level and metabolism of flavanols in humans and their biological activity in vivo. Free Radic Biol Med 50:237–244

Patil BS, Jayaprakasha GK, Chidambara Murthy KN, Vikram A (2009) Bioactive compounds: historical perspectives, opportunities, and challenges. J Agric Food Chem 57:8142–8160

Ramprasath VR, Eyal I, Zchut S, Jones PJH (2013) Enhanced increase of omega-3 index in healthy individuals with response to 4-week n-3 fatty acid supplementation from krill oil versus fish oil. Lipids Health Dis 12:178

Reddy VC, Vidya Sagar GV, Sreeramulu D, Venu L, Raghunath M (2005) Addition of milk does not alter the antioxidant activity of black tea. Ann Nutr Metab 49:189–195

Rein MJ, Renouf M, Cruz-Hernandez C, Actis-Goretta L, Thakkar SK, da Silva Pinto M (2013) Bioavailability of bioactive food compounds: a challenging journey to bioefficacy. Br J Clin Pharmacol 75:588–602

Renouf M, Marmet C, Guy P, Fraering A-L, Longet K, Moulin J, Enslen M, Barron D, Cavin C, Dionisi F, Rezzi S, Kochhar S, Steiling H, Williamson G (2010) Nondairy creamer, but not milk, delays the appearance of coffee phenolic acid equivalents in human plasma. J Nutr 140:259–263

Ribnicky DM, Roopchand DE, Oren A, Grace M, Poulev A, Lila MA, Havenaar R, Raskin I (2014) Effects of a high fat meal matrix and protein complexation on the bioaccessibility of blueberry anthocyanins using the TNO gastrointestinal model (TIM-1). Food Chem 142:349–357

Rocha RA, de la Fuente B, Clemente MJ, Ruiz A, Vélez D, Devesa V (2013) Factors affecting the bioaccessibility of fluoride from seafood products. Food Chem Toxicol 59:104–110

Rossi L, ten Hoorn JWMS, Melnikov SM, Velikov KP (2010) Colloidal phytosterols: synthesis, characterization and bioaccessibility. Soft Matter 6:928–936

Roura E, Andrés-Lacueva C, Estruch R, Lourdes Mata Bilbao M, Izquierdo-Pulido M, Lamuela-Raventós RM (2008) The effects of milk as a food matrix for polyphenols on the excretion profile of cocoa (−)-epicatechin metabolites in healthy human subjects. Br J Nutr 100:846–851

Roura E, Andrés-Lacueva C, Estruch R, Mata-Bilbao ML, Izquierdo-Pulido M, Waterhouse AL, Lamuela-Raventós RM (2007) Milk does not affect the bioavailability of cocoa powder flavonoid in healthy human. Ann Nutr Metab 51:493–498

Scheepens A, Tan K, Paxton JW (2010) Improving the oral bioavailability of beneficial polyphenols through designed synergies. Genes Nutr 5:75–87

Scholz S, Williamson G (2007) Interactions affecting the bioavailability of dietary polyphenols in vivo. Int J Vitam Nutr Res 77:224–235

Schroeter H, Holt RR, Orozco TJ, Schmitz HH, Keen CL (2003) Nutrition: milk and absorption of dietary flavanols. Nature 426, 787–788 (discussion 788)

Schweiggert RM, Kopec RE, Villalobos-Gutierrez MG, Högel J, Quesada S, Esquivel P, Schwartz SJ, Carle R (2013) Carotenoids are more bioavailable from papaya than from tomato and carrot in humans: a randomised cross-over study. Br J Nutr 1–9

Serafini M, Bugianesi R, Maiani G, Valtuena S, De Santis S, Crozier A (2003) Plasma antioxidants from chocolate. Nature 424:1013

Serra A, Macià A, Rubió L, Anglès N, Ortega N, Morelló JR, Romero M-P, Motilva M-J (2013) Distribution of procyanidins and their metabolites in rat plasma and tissues in relation to ingestion of procyanidin-enriched or procyanidin-rich cocoa creams. Eur J Nutr 52:1029–1038

Shen Z, Apriani C, Weerakkody R, Sanguansri L, Augustin MA (2011) Food matrix effects on in vitro digestion of microencapsulated tuna oil powder. J Agric Food Chem 59:8442–8449

Tzeng C-W, Yen F-L, Wu T-H, Ko H-H, Lee C-W, Tzeng W-S, Lin C-C (2011) Enhancement of dissolution and antioxidant activity of kaempferol using a nanoparticle engineering process. J Agric Food Chem 59:5073–5080

Van der Burg-Koorevaar MCD, Miret S, Duchateau GSMJE (2011) Effect of milk and brewing method on black tea catechin bioaccessibility. J Agric Food Chem 59:7752–7758

Victoria-Campos CI, de Ornelas-Paz J, Yahia EM, Failla ML (2013) Effect of the interaction of heat-processing style and fat type on the micellarization of lipid-soluble pigments from green and red pungent peppers (Capsicum annuum). J Agric Food Chem 61:3642–3653

Walde CM, Drotleff AM, Ternes W (2013) Comparison of dietary tocotrienols from barley and palm oils in hen's egg yolk: transfer efficiency, influence of emulsification, and effect on egg cholesterol. J. Sci, Food Agric

Walsh KR, Zhang YC, Vodovotz Y, Schwartz SJ, Failla ML (2003) Stability and bioaccessibility of isoflavones from soy bread during in vitro digestion. J Agric Food Chem 51:4603–4609

Welch IM, Cunningham KM, Read NW (1988a) Regulation of gastric emptying by ileal nutrients in humans. Gastroenterology 94:401–404

Welch IM, Davison PA, Worlding J, Read NW (1988b) Effect of ileal infusion of lipid on jejunal motor patterns after a nutrient and nonnutrient meal. Am J Physiol 255:G800–806

Whitley AC, Sweet DH, Walle T (2005) The dietary polyphenol ellagic acid is a potent inhibitor of hOAT1. Drug Metab Dispos 33:1097–1100

Wu S, Sun K, Wang X, Wang D, Wan X, Zhang J (2013) Protonation of epigallocatechin-3-gallate (EGCG) results in massive aggregation and reduced oral bioavailability of EGCG-dispersed selenium nanoparticles. J Agric Food Chem 61:7268–7275

Xie X, Tao Q, Zou Y, Zhang F, Guo M, Wang Y, Wang H, Zhou Q, Yu S (2011) PLGA nanoparticles improve the oral bioavailability of curcumin in rats: characterizations and mechanisms. J Agric Food Chem 59:9280–9289

Yliperttula M, Urtti A (2009) Nanotechnology for improved drug bioavailability. In: Van de Waterbeemd H. Testa B. (eds) Drug bioavailability: estimation of solubility, permeability, absorption and bioavailability, 2nd edn. Wiley-VCH, p 597–604

Yu H, Shi K, Liu D, Huang Q (2012) Development of a food-grade organogel with high bioaccessibility and loading of curcuminoids. Food Chem 131:48–54

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