Chapter 24

Encapsulation of Bioactives

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24.1 Introduction

Food bioactives are physiologically active components in food or dietary supplements of plant or animal origin that have a role in health beyond basic nutrition. The addition of bioactive components to foods, particularly those foods that are consumed as part of the normal diet of target populations, offers opportunities for improving the health and well-being of consumers. The interest of the food industry in these functional foods has resulted in the development of a new generation of food products with enhanced levels of food components that have potential health benefits (Schmidl and Labuza 2000; Hilliam 2000; Heasman and Mellentin 2001; Augustin and Clarke 2004).

The delivery of bioactives through food is a major challenge. Many bioactives are prone to degradation, and thus there is a need to protect them throughout their shelf-life as both an ingredient and in fortified food products, without compromising the sensory properties of the food. In addition, the bioactivity needs to be maintained so it is available when consumed in order to have a physiological function when delivered to its particular target site within the body. All these requirements place stringent demands on using food to deliver bioactives. Often, these demands cannot be met by direct addition of a bioactive to food, as it needs to be protected prior to its release.

Microencapsulation has been used for protection and delivery of bioactives in food applications. In encapsulation, components (referred to as the core or active) are packaged within a secondary material (referred to as the wall material or the encapsulant) and delivered in small particles.

This chapter considers the issues relating to the delivery of bioactives through foods. The choice of materials for encapsulation of bioactives, the formulation of the encapsulated delivery system and the processes used for their manufacture are discussed. Examples of materials and processes used for the manufacture of encapsulated fat-soluble and water-soluble bioactives and encapsulated probiotics are given. The effectiveness of various encapsulated delivery systems for protection of bioactive ingredients and new trends in encapsulation technology are covered. The

requirements for effective encapsulation and the material states that may be used are very wide. Obvious connections can be drawn between the processes described in this chapter and the principles outlined in others (in particular Chapters 3, 5, 9, 15 and 23).

24.2 Issues Relating to Addition of Bioactives to Food

The range of food components now considered as bioactives include vitamins, minerals, functional lipids, probiotics, amino acids, peptides and proteins, phytosterols, phytochemicals and antioxidants (Wildman 2001). Their structure and function vary widely and are important considerations when adding them to food. The health aspects of bioactive ingredients and functional foods are not covered here as they are beyond the scope of this chapter.

Many bioactives are unstable. Irrespective of the form they are added to food, it is essential that they be stabilized prior to addition to food, during the food manufacturing process and throughout the food product's shelf-life. When choosing the food vehicle for addition of a chosen bioactive, it is important to consider its solubility in the food matrix and its interactions with other ingredients in the food formulation. The incorporation of bioactives can alter flavour, odour and texture of foods. As consumers only accept food products with good sensory appeal, the successful addition of bioactives into a range of functional food products must not compromise food quality. Furthermore, as the bioactive food component is selected for its specific physiological function, it is important that it is bioavailable when the food is consumed.

24.2.1 Solubility of Bioactives

When considering the addition of a bioactive to food, it is useful to classify them as oil-soluble (e.g., polyunsaturated fatty acids, carotenes, lycopene), water-soluble (e.g., anthocyanins, proteins and peptides), or water/oil dispersible components (e.g., probiotics). Bioactives may be added directly to food if they are in a compatible format with the food matrix and provided their direct addition does not impact negatively on food quality or the bioavailability of the bioactive. When the solubility in a food matrix is limiting, its hydrophilicity/lipophilicity may be modified to enable improved incorporation. An example is the conversion of free plant sterols to fatty acid esters in order to make them more oil-soluble and readily incorporated into spreads (Deckere de and Verschuren 2000).

24.2.2 Stability of Bioactives

Bioactive ingredients are extracted from plant and animal sources and are provided to food manufacturers as liquid extracts, concentrates or powders. Generally, once the bioactive is extracted from its natural source, it is more susceptible to degradation.

It is well-known that vitamins A and D are sensitive to oxygen, light, and the presence of oxidizing agents. Long-chain polyunsaturated oils are susceptible to

oxidation if not protected against light, oxygen and/or trace metal ions such as iron or copper (Frankel et al. 2002). Anthocyanins and polyphenols are not stable to heat during juice processing (Fang et al. 2006). The stability of anthocyanins varies depending on the processing conditions, and the presence of other components in their new environment (Kirca et al. 2006).

Food manufacturers have generally added higher levels of bioactives than required to compensate for losses during processing and storage. To ensure that the level of addition claimed is maintained throughout the shelf-life of the food, overages of 5%–50% of beta-carotene and vitamins C and E have been added to dairy products (Elliot 2001).

24.2.3 Interactions of Bioactives with Other Food Components

When a bioactive is added to food, it may react with other food components causing degradation and loss of bioactivity. Even when the bioactive is not sensitive to degradation (e.g., minerals), the addition of the component at a level higher than that normally found in the target food vehicle alters its properties. Reformulation and/or modified processing conditions may be necessary. For example, the addition of calcium into foods containing proteins can cause precipitation. Reformulation approaches, which have been used to overcome some of the heat stability problems with mineral fortified dairy products, have included the use of phosphates in Ca-fortified milks (Williams et al. 2005) and caseins to Fe-fortified products (Sher et al. 2006). Phosphates and caseins bind divalent metal ions, thus reducing the levels of the free calcium or iron, which are primarily responsible for the protein precipitation problems.

24.2.4 Taste and Odour

Many bioactives have an undesirable taste and odour. Peptides are known for their bitter taste, mineral salts for their metallic tastes and marine oils rich in omega-3 fatty acids for fishy taste and odour. Further, the addition of soluble iron salts to foods catalyses the oxidation of fats and amino acids and imparts undesirable metallic tastes to foods (Zimmermann 2004; Yang and Lawless 2006). A variety of added ingredients (e.g., sugar, flavours) have been used to mask these tastes, but with limited success.

24.2.5 Bioavailability

The bioactive needs to be in a bioavailable form when consumed. Its addition must also not affect the bioavailability of other desirable food components, and interaction with other food components can decrease bioavailability. It is important to select the appropriate form of the bioactive and the formulation for delivery in order to maintain bioavailability.

24.3 Encapsulation of Bioactives

The requirements discussed above have led to the delivery of bioactives in microencapsulated forms. Encapsulation technology alleviates many of the problems encountered with direct addition of bioactives (Pszczola 1998; Augustin 2001; Zimmermann 2004) because the components are entrapped within the matrix of a coating material (e.g., entrapment of oils within a glassy carbohydrate matrix) or encased within a protective coating (e.g., oil droplets surrounded by an interfacial layer of protein). A schematic representation of encapsulated bioactives is given in Figure 24.1. Encapsulation has the potential to isolate the bioactive until its release is triggered.

Microencapsulation technology is well developed and commercialized in the chemical, pharmaceutical, cosmetic and printing industries. In the food industry, it is used to mask unpleasant tastes and protect sensitive ingredients from degradation through reactions with its environment during storage, and from other components when it is introduced into a food matrix (Gibbs et al. 1999; Augustin et al. 2001; Pszczola 2003). The ability to control the release of the encapsulated ingredient enables suppliers to increase the effectiveness of their ingredients whilst lessening the need for over addition, and to widen their range of applications (Gouin 2004).

Microencapsulation technology has been traditionally used in the food industry for flavour encapsulation where flavours are stabilized and their release controlled (Madene et al. 2006). Microencapsulation has also been used to enable the incorporation of sensitive bioactive component in fortified foods, while ensuring that the taste, aroma, or texture of food is not adversely affected (Pszczola 1998; Brazel 1999; Augustin et al. 2001). Microencapsulation can reduce off-flavours contributed by certain vitamins and minerals, permit time-release of the nutrients, enhance stability to extremes in temperature and moisture, and reduce undesirable chemical interactions with other ingredients.

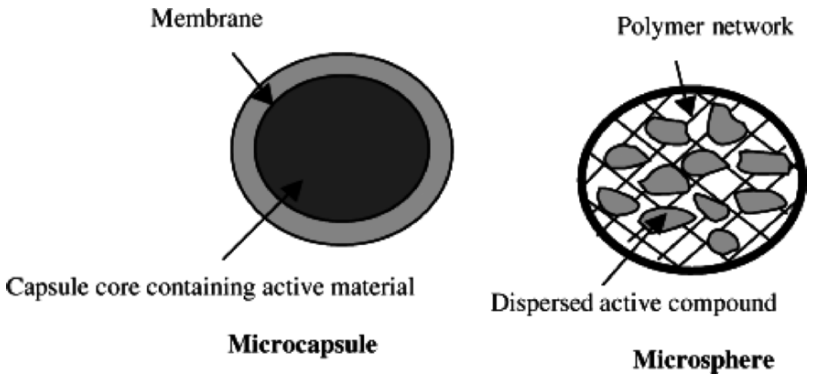

Figure 24.1. A schematic representation of encapsulated bioactives. (Reprinted with permission from Madene et al. 2006.)

24.3.1 Considerations for Design of the Delivery System

The specific requirements of the encapsulant material and the delivery system have to be considered. This includes protecting the bioactive throughout its storage as an ingredient, through the food manufacturing process, and during the shelf-life of the food product. In order for the bioactive to survive food processing conditions, it is important to ensure that the core is protected from harsh processing environments and that the release of the core is not triggered prematurely. The conditions during gastrointestinal transit of the food should also to be taken into account to deliver the bioactive to the desired site of the body to achieve its potential health benefits (Bell 2001).

The development of a successful encapsulation system for a target application requires knowledge about the stability of the chosen bioactive (core); the properties of the materials used for encapsulation (encapsulant) and the suitability of the delivery system (microcapsule) for its final application. Table 24.1 gives a summary of important considerations.

Table 24.1. Properties of the core, encapsulant material and microcapsule of importance in the design of encapsulated bioactives.

Component	Properties
Core	Bioactivity of the material
	Solubility (hydrophilicity/lipophilicity)
	Stability to environmental conditions (e.g., moisture, heat, pH, salts, light, enzymes)
	Taste
	Propensity to interact with other food components
Encapsulant material	Solubility
	Viscosity
	Stability to pH, salts, temperature, shear, enzyme degradation
	Film forming and emulsification properties
	Regulatory status for food application
Microcapsule	Format for delivery (i.e., liquid or powder)
	Storage stability
	Stability to different process conditions
	Release properties
	Particle size
	Payload (bioactive core loading)
	Cost of production

24.3.2 Encapsulant Materials

Encapsulant materials can be selected from a wide range of natural or synthetic materials depending on the properties desired in the final microcapsule (Table 24.2). The encapsulant materials in food are more restricted to natural food components (e.g. proteins, sugars, starches, gums, lipids, cellulosic material) and other ingredients

that have Generally Regarded As Safe (GRAS) status (e.g., cyclodextrin, chitosan, low-molecular weight emulsifiers such as Tweens, mineral salts, etc.). They can be used alone or in combination to achieve the desired functionality. The composition can significantly influence the functional properties of the final microcapsule as can the choice of processing technologies used. Cost also needs to be considered.

Neutral taste and odour, low viscosity, good film forming, gelling and barrier properties are some of the desired characteristics of encapsulant materials. The functional properties of food biopolymers (water binding, gelling, emulsifying) lend themselves to the development of a wide range of microstructures that can be used for delivery of bioactives. A good encapsulant protects the core from degradation during processing and storage and also masks any undesirable taste and odour associated with the bioactive core when added into a food product. Identification of the storage requirements and processing needs, as well as the mechanisms required for release of the core material (e.g., pressure-based, dissolution-based or melting-based triggers) are important. These are influenced by the mechanical strength of the capsule wall and its compatibility with the target food product. Nutritional value, sensory properties and aesthetic properties are also important considerations (Brazel 1999; Gibbs et al. 1999). Thus, the material properties of the capsule such as the permeability of the encapsulant, its resistance to conditions encountered (e.g., shear, heat, pH shift, light, enzyme, other ingredients) during processing and gastrointestinal tract transit have to be designed to enable the desired control over the release of the bioactive.

Table 24.2. Materials used for encapsulation of bioactives for food applications.

Material class	Types of materials
Proteins	Milk proteins—caseins and whey proteins,
	Soy proteins
	Wheat proteins
	Egg proteins
	Zein
	Hydrolyzed proteins
Carbohydrates	Sugars—fructose, galactose, glucose, maltose, sucrose, oligosaccharide, corn syrup solids, dried glucose syrup
	Starch and starch products—maltodextrins, dextrins, starches, resistant starch, modified starches
	Gums—agar, alginates, carrageenan, gum acacia, gum arabic, pectin,
	Carboxymethyl cellulose
	Chitosan
	Cyclodextrins
Lipids	Natural fats and oils
	Fractionated fats
	Mono- and diglycerides
	Phospholipids
	Glycolipids
	Waxes—beeswax, carnauba wax

The limited range of suitable encapsulant materials allowed for food use still remains the biggest challenge in material selection, especially when more sophisticated properties are required by food manufacturers and consumers. Modification of existing food grade materials to achieve differentiated encapsulant functionality may be required to achieve new properties in microcapsules and improved performance of microencapsulated ingredients. A new generation of encapsulant systems has emerged for food applications. Some of the new developments in materials for microencapsulation involve capitalizing on the interactions between biopolymers or modification of the native food ingredient either chemically or physically to achieve new functional properties not present in the native food ingredients. Examples are the development of protein–polysaccharide complexes (Schmitt et al. 1998) and Maillard reaction conjugates (Augustin et al. 2006).

24.3.3 Processing of Microcapsules

The selection of the method or microencapsulation process depends on the properties of the core and the coating materials, the release mechanism desired, process type, capsule morphology and particle size. Most of the processes have been adapted from the pharmaceutical and chemical industries. The use of low-cost materials and manufacturing processes for encapsulation has been a significant challenge as food products generally have lower profit margins compared to pharmaceutical and chemical products. Both physical and chemical processes are available to encapsulate a range of bioactive ingredients (Table 24.3). The selection of the method depends on the required format and inherent properties of the bioactive core and the encapsulant materials used. Selected processes used in the food industry are discussed below.

Table 24.3. Methods used for microencapsulation.

Physical processes	Chemical processes
Spray-drying	Simple coacervation
Spray-chilling	Complex coacervation
Fluidized bed coating	Solvent evaporation
Centrifugal extrusion	Liposomes
Spinning disk coating	Chemical adsorbents
Pressure extrusion	Inclusion complexation
Hot melt extrusion	
Use of supercritical fluids	

24.3.3.1 Spray Drying

Spray drying is the most commonly used method in the food industry. Bioactive ingredients microencapsulated by this method include fats and oils, flavours, essential oils and other oil-soluble bioactives. Water-soluble bioactives can also be encapsulated by spray drying, where the encapsulant forms a matrix structure rather than a film surrounding the core. This process typically involves the dispersion of the core material into a solution of the encapsulant (e.g., protein, carbohydrate) and atomization of the mixture into the drying chamber. This leads to evaporation of the solvent

(water) and the formation of powder microcapsules. For the encapsulation of oils and oil-soluble bioactives, an emulsion is prepared prior to spray drying. The advantage of the spray-drying process is that it is cheap and it can be operated on a continuous basis. Proper adjustment and control of the processing conditions enables the tailoring of desirable powder properties such as particle size, moisture content, bulk density, flowability, dispersibility, appearance and structural strength (Reineccius 2001).

24.3.3.2 Spray Chilling

Spray chilling is a process that involves dispersing the core into a warm, liquefied coating material and spraying through a heated nozzle into a controlled environment, where the encapsulant solidifies to form the microcapsule particles (Lamb 1987). The process is performed in equipment similar to that used in spray drying except that the process air is not heated. Encapsulant materials commonly used in this process are vegetable oils and waxes or their derivatives (e.g., hydrogenated or fractionated vegetable oils with high melting points). This technique is commonly used for microencapsulation of water-soluble bioactive core materials such as vitamins and minerals to minimize their solubility in the water environment, or for encapsulation of bioactives for temperature release applications (Anon 1981).

24.3.3.3 Fluidized Bed Coating

Fluidized bed coating is accomplished by suspending, or fluidizing, particles of a core material in an upward stream of air and applying an atomized coating material to the fluidized particles (Figure 24.2). The core and the encapsulant material must be stable under the processing conditions applied during the application and drying

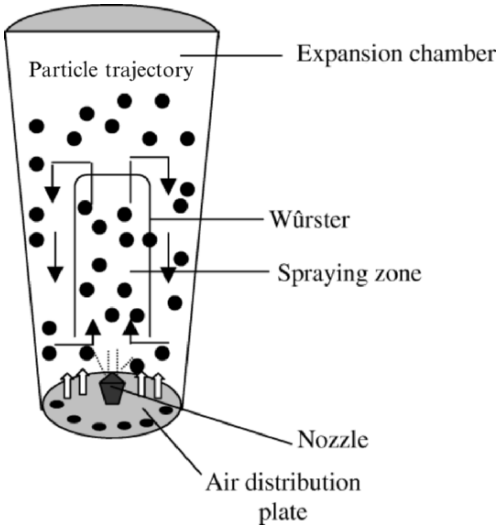

Figure 24.2. Fluidized bed coating process. (Reprinted with permission from Madene et al. 2006.)

of the coat. The coating may be a solution, dispersion, emulsion, fat or other film forming material. Fluidized bed coating has been used to enhance or tune the properties of bioactive ingredients, to increase shelf-life, mask undesirable taste, improve handling, control release, and improve aesthetics, taste and color (Dewettinck and Huyghebaert 1999). It is used for production of nutritional supplements such as vitamins B and C, ferrous sulfate, ferrous fumarate, sodium ascorbate, potassium chloride and a variety of vitamin and mineral premixes. Spray-dried oil-in-water emulsions can be given a secondary coating to improve core stability with a fluid bed coating process (Turchiuli et al. 2005).

24.3.3.4 Extrusion

Extrusion processes involve the application of pressure to a mass to make it flow through an orifice or die under controlled conditions. An extrusion device for encapsulation consists of a droplet generator and a droplet hardening bath (Rabiskova 2001). Microencapsulation by extrusion takes place at very high temperatures, and involves projecting an emulsion of the core and coating material through a die at high pressure. This method has been traditionally used in flavour encapsulation. Its potential for use in bioactive delivery was demonstrated by work on encapsulation of sunflower oil (as a model lipophilic bioactive) in a potato starch matrix by extrusion (Yilmaz et al. 2001).

24.3.3.5 Centrifugal Extrusion Encapsulation

Centrifugal extrusion encapsulation, or coaxial extrusion processing, is a liquid co-extrusion process that uses an assembly of cylindrical nozzles consisting of concentric orifices located at the outer circumference of a rotating cylinder (Figure 24.3).

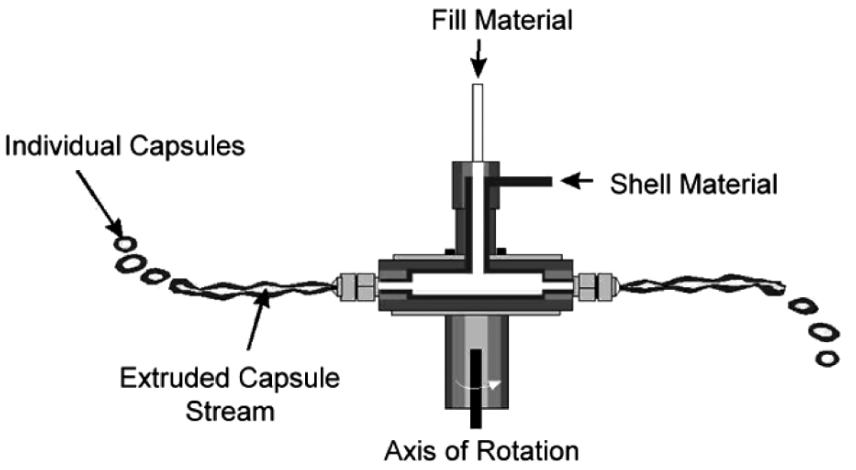

Figure 24.3. Centrifugal extrusion encapsulation. (Reprinted with permission from Southwest Research Institute, USA.)

The process involves a liquid bioactive core being pumped through the inner orifice, and the liquid encapsulant material pumped through the outer orifice, which are both located in the cylindrical head. When co-extruded simultaneously, a co-extruded liquid jet of the bioactive core material is surrounded by the encapsulant. As the cylindrical head rotates the extruded liquid jet breaks into droplets and forms the microcapsule. The microcapsules are hardened by drying, cooling, chelation, gelation, chemical reaction, or cross-linking, depending on the nature of the encapsulant material (Vasishtha and Schlameus 2001). Centrifugal extrusion encapsulation requires the bioactive core to be in a liquid form at the time of production.

24.3.3.6 Spinning-Disk Coating

Spinning-disk coating or centrifugal suspension-separation coating is a physical coating process that involves the suspension of solid core particles in a liquid encapsulating material (Sparks et al. 1995) (Figure 24.4). The encapsulant formulation can be a solution, suspension or melt directly applied without solvents. The bioactive core suspension is then passed over a rotating disk under very controlled process condition to form a thin film around the core particles. Excess film material is separated from the coated particles and recycled. This process can coat particles from about 20 microns to several millimeters in diameter (Labell 2002).

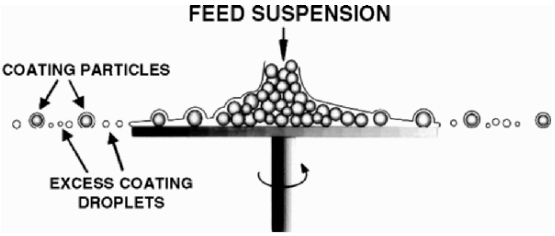

Figure 24.4. Spinning-disk coating. (Reprinted with permission from Particle Coating Technologies, USA.)

24.3.3.7 Use of Supercritical Fluids

Supercritical fluids can be used for the encapsulation of heat-sensitive cores. This solvent free-method can be used for the production of bioactive loaded microparticles as the encapsulant material is plasticized in the supercritical carbon dioxide, which is mixed with the core. In microencapsulation by rapid expansion of supercritical solutions, the pressurized supercritical fluid containing the encapsulant and core is sprayed through a nozzle. This leaves a particulate material containing the core and has been used for encapsulation of food grade ingredients such as vitamin C and xylitol (Gouin 2004). Supercritical fluid mixing has been used for the production of a protein encapsulated in poly(DL-lactic acid) microparticles in which the activity of the protein is retained, demonstrating the potential use of supercritical fluids for the controlled release of proteins and peptides (Whitaker et al. 2005). By altering depressurization conditions, particles with different morphologies may be obtained.

24.3.3.8 Complex Coacervation

Coacervation is the separation of two liquid phases in colloidal systems, where the more concentrated phase is known as the coacervate. Complex coacervates are formed by combining two oppositely charged biopolymers under controlled conditions. This causes associative phase separation (Kruif et al. 2004). Typically, protein and polysaccharide mixtures are used and the capsule is hardened by using a cross-linker (Figure 24.5). It is the hardening of the shell that remains the greatest challenge, especially for food applications. Glutaraldehyde is usually added for cross-linking in pharmaceutical applications but not in food applications. Alternative cross-linking systems are now being examined and the use of plant phenolics as cross-linking agents has been demonstrated for cross-linking gelatin gels and gelatin-pectin coacervate microparticles (Strauss and Gibson 2004).

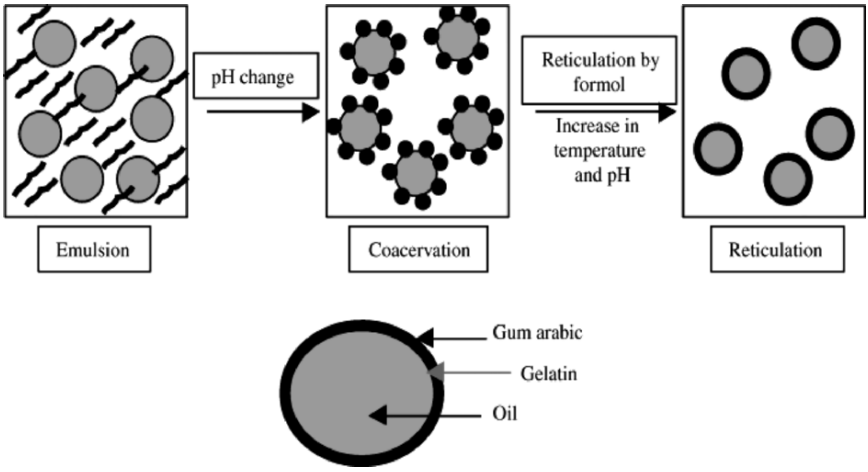

Figure 24.5. Principle of complex coacervation method. (Reprinted with permission from Madene et al. 2006.)

The advantage of complex coacervates is that high payloads can be obtained. Chitosan/alginate coacervates have been reported to encapsulate up to 87% shark liver oil, which is rich in omega-3 fatty acids (Peniche et al. 2004). Microspheres of carboxymethyl chitosan/alginate hardened with calcium chloride have been used for encapsulation of up to 80% bovine serum albumin (Zhang et al. 2004).

24.3.3.9 Inclusion Complexation

In inclusion complexation, a core is encapsulated by complexation (e.g., cyclodextrin). A supramolecular assembly is formed because of the noncovalent inclusion of an apolar core (e.g., oil) of suitable size within the hydrophobic cavity of the cyclodextrin. An example of inclusion complexation is the interaction between cyclodextrin and lycopene (Mele et al. 2002). The inclusion complex increases the water

dispersibility of the lipophilic molecules, controls their release and protects them from the environment.

24.3.3.10 Liposomes

Liposomes are spherical layered vesicles consisting of one or more concentric layers of lipids formed from dispersion of polar lipids in aqueous solvents. Liposomes have been used for the delivery of drugs in the pharmaceutical industry and also have widespread applications in the agriculture and food industry (Taylor et al. 2005). Liposomes are usually prepared with phospholipids, but glycolipids also possess liposome-forming properties (Herslof 2000). Depending on the conditions of their preparation, unilamellar or multilamellar liposomes can be formed. The entrapment of sensitive oil and water-soluble bioactives in liposomes protects them prior to their release, and the size, composition and stability of the liposomes can be tailored to meet the functional requirements of the system. Liposomes can offer a versatile approach, for encapsulation of both oil-soluble and water-soluble bioactives, with opportunities to induce controlled release of the bioactive core. It has been used in the food industry for protection of enzymes, vitamins, minerals, bacteriocins and antimicrobials, but still remains an expensive process (Zeisig and Cammerer 2001).

24.3.3.11 Multiple Emulsions

Multiple emulsions are compartmentalized liquid dispersions in which the inner dispersed phase is separate from the outer liquid phase by a middle layer of another phase. Water-in-oil in water (*W/O/W*) or oil-in-water in oil (*O/W/O*) may be prepared. Double emulsions can be used to deliver both oil and water-soluble bioactives, and are suited for controlled and sustained release of the active core encapsulated in the internal phase of the double emulsion. Conventionally, double emulsions are made with low-molecular-weight surfactants which can limit their long-term stability. This has been improved with the use of natural biopolymers to stabilize the system (Benichou et al. 2004). Alternatively, the oil phase of double emulsions may be modified to provide control of the release of an encapsulated hydrophilic bioactive. Fats with different melting points used in *W/O/W* emulsions containing L-tryptophan as a marker compound, have different capacities to perform as hydrophobic barriers. The release of L-tryptophan was slowed with increasing melting point of the lipid phase (Weiss et al. 2005). The structure of the double emulsions can be preserved after spray drying, as demonstrated with a double emulsion containing orange oil (Edris and Bergenståhl 2001). This allows double emulsions to be produced in both liquid and powder formats.

Multiple emulsions can also be formed by mixing an oil-in-water emulsion with a thermodynamically incompatible biopolymer mixture. Depending on the formulation and conditions of preparation, oil-in-water-in-water or mixed oil in water/water-in-water multiple emulsions may be formed. These have potential for the controlled delivery of a range of bioactives (Kim et al. 2006).

24.4 Encapsulated Bioactive Delivery Systems

Encapsulated delivery systems are developed with a specific purpose for each target application. To date, most research has been directed at stabilizing extracted bioactives and delivering the required levels in food. Their ultimate delivery to a target site depends on the core, the material and the methods used for encapsulation as well as the food vehicle used for delivery. Although there have been many technological advances there are still limitations to the use of microencapsulated ingredients in some applications.

24.4.1 Encapsulated Delivery Systems for Lipids

Many functional lipids including polyunsaturated lipids such as omega-3 fatty acids, conjugated linoleic acids and lipid-soluble bioactives such as carotenoids, coenzyme Q10, tocopherols and some vitamins are sensitive to oxidation. If not adequately protected from the environment, they can degrade very rapidly. This results in the development of off-flavours and reduced bioactivity. Many types of materials have been used to encapsulate sensitive lipids to isolate them from oxygen and minimize their interactions with trace metal ions (e.g., Fe) that catalyze their oxidation. Lipids may be trapped in glassy matrices, stabilised in emulsion systems with low molecular weight surfactants or film forming biopolymers, or encapsulated in liposomes or cyclodextrin.

24.4.1.1 Glassy Matrices

The ability of glassy matrices to encapsulate lipophilic bioactive molecules makes them useful for the delivery of oils and oil-soluble bioactives. However, the efficiency of encapsulation and the degree of protection offered varies with the formulation. Some authors suggest that increased molecular mobility as the temperature is raised above the glass transition temperature, leads to an increase in oxidation of encapsulated lipid components (Gejl-Hansen and Flink 1977). Shimada et al. (1991) found that above the glass transition temperature, encapsulated methyl linoleate was released from a lactose-gelatin matrix, resulting in its rapid oxidation. Labrousse et al. (1992) demonstrated that oxidation of methyl linoleate was arrested in sugar-gelatin glassy matrices but that when the matrix collapsed, rapid oxidation occurred. However, others have found that molecular mobility did not influence oxidation rates of flaxseed oil (Grattard et al. 2002). Others have shown that there was only partial protection of the oils as oxygen can permeate a glassy food matrix (25% sucrose, 45% maltodextrin, 5% gelatin and 25% rapeseed oil), where the limiting factors were oxidation at low temperature and oxygen diffusion at higher temperatures (Orlien et al. 2006).

24.4.1.2 Gels and Beadlets

Gelatin beadlets have been used for delivery of β-carotene (Pszczola 2003). Recently, multicore carotenoid beadlets have been prepared by spray drying a dispersion of

carotenoids in gelatin, pectin and medium chain fatty acid glycerides (Sadano and Sonoda 2002), and are protected against degradation when encapsulated in these matrices.

24.4.1.3 Emulsion Systems

Oil-in-water emulsions lend themselves readily to the delivery of oils and oil-soluble bioactives. The surfactant or biopolymer provides a means of isolating and protecting the lipophilic cores. Many types of materials with emulsifying capacity have been used to encapsulate oils and oil-soluble bioactives in single and multiple emulsion systems. Multilayered interfaces have also been used to improve the robustness of microcapsules.

Primary Emulsions

Proteins (e.g., milk proteins, gelatin, soy proteins) stabilize oil-in-water emulsions. Protein encapsulants have been successfully used for protecting sensitive oils against oxidation (Hu et al. 2003). Caseins are effective for stabilizing spray-dried emulsions of fish oil, with more aggregated forms of the caseins providing more protection to the oil against oxidation (Keogh et al. 2001). The proteins can inhibit oxidation either at the interface of the emulsion droplet or in the aqueous phase (McClements and Decker 2000; Faraji et al. 2004).

Although the protein component is needed for emulsification, sugars are often added to the mix. The oxidative stability of spray-dried fish oil emulsions was found to increase with increasing dextrose equivalence (DE) of the carbohydrate used in combination with caseinate (Hogan et al. 2003; Kagami et al. 2003). This may be due to the increased microencapsulation efficiencies with increasing DE of the carbohydrate as observed in soy oil powders stabilized by blends of caseinate and carbohydrates (Hogan et al. 2001) or the antioxidant properties of small sugars in fish oils (Faraji and Lindsay 2005). Higher microencapsulation efficiencies might be expected to impart improved oxidative stability of sensitive lipids. However, this does not always appear to be the case. The stability of freeze-dried fish oil emulsions containing sodium caseinate and lactose was not related to microencapsulation efficiency (Heinzelmann et al. 2000).

The encapsulating properties of a protein may be improved by conjugation of sugars to proteins. The Maillard reaction between a reducing sugar and a free amino group has been utilized to increase the emulsifying capacity of proteins. In addition, Maillard reaction products have antioxidant qualities and have been found to be superior encapsulants compared to the original protein for protecting fish oils and spray dried emulsions from oxidation (Augustin et al. 2006).

Other protein systems have been successfully used for encapsulation. For example, conjugated linoleic acid has been microencapsulated using whey proteins (Jimenez et al. 2004). A whey protein bead delivery system was prepared by emulsification of soybean oil containing retinol with pre-denatured whey protein followed by the addition of calcium ions to induce gelation. The encapsulant system protected

the retinol from enzymatic degradation at stomach pH and could be released on pancreatic digestion (Beaulieu et al. 2002).

The use of a modified starch, corn starch sodium octenyl succinate derivative, for encapsulation of sea buckthorn kernel oil (containing polyunsaturated fatty acids, tocopherols, tocotrienols, plant sterols and carotenoids) by spray-drying was found to improve oil stability. Better protection was afforded when the starch encapsulant was stored in its glassy state (Partanen et al. 2002). Methylcellulose and hydroxymethycellulose in combination with soy lecithin enabled the production of 40% (*W/W*) fish oil powders with improved stability (Kolanowski et al. 2004).

Double Emulsions

Water-dispersible microcapsules have also been prepared by spray-drying *W/O/W* emulsions containing carotenoids. These emulsions were formulated with combinations of low molecular weight emulsifiers and biopolymer blends of various gums. Microencapsulation efficiency and protection of carotenoids against degradation was significantly influenced by the formulation used (Rodríguez-Huezo et al. 2004). A double emulsification process, followed by heat gelation of protein microcapsules, holds promise for controlled core-release in food systems (Lee and Rosenberg 2000). Oil-in-water-in-oil emulsions containing fish oils that were subsequently gelled by heat or treated with transglutaminase to cross-link the proteins in the capsules exhibited improved oxidative stability and provided greater resistance to pepsin attack compared to nonencapsulated oils (Cho et al. 2003).

An alternative to the traditional *O/W/O* or *W/O/W* emulsions is the *O/W/W* format. The addition of a whey–protein oil-in-water stabilized emulsion to an aqueous two-phase system (comprising heat denatured whey protein and high methoxy pectin) resulted in the formation of such an emulsion. This was subsequently gelled with calcium ions. It has been suggested that these novel structures can provide both encapsulation and controlled release (Kim et al. 2006).

Multilayered Oil-in-Water Emulsions

Improved stability of lipophilic bioactives may be obtained by tailoring the interfacial membranes of oil droplets. Rosenberg and Lee (2004) coated a primary whey protein–based oil-in-water emulsion containing paprika oleoresin (as the model core) with calcium alginate to enhance the stability and control the core release.

Stabilization of oil droplets may also be achieved by engineering multilayered interfacial membranes by adsorption of oppositely charged biopolymers, using a layer-by-layer deposition process. Secondary emulsions, formed by the dilution of a primary anionic oil-in-water emulsion made with a low molecular weight surfactant (lecithin), with an aqueous chitosan solution. The secondary emulsions containing oil droplets stabilized by lecithin-chitosan coated membranes had increased oxidative stability and were more resistant to aggregation when exposed to heat, freeze–thaw cycling and high calcium concentration compared to the primary emulsions (Ogawa et al. 2003). Tertiary emulsions may also be formed by deposition of biopolymers of opposite charge as in the formation of lecithin–chitosan–pectin

oil-in-water emulsion droplets (Ogawa et al. 2004). By the addition of multilayers, the interfacial membranes of the primary emulsion droplet may be modified to provide the desired protection—that is, increase stability of the core or control its release.

The structure of the multilayered emulsions may be preserved during spray-drying, enabling the delivery of emulsions with multilayered interfaces in a powder format. Spray-dried tuna oil powders made from emulsions containing oil droplets with lecithin–chitosan membranes, with added corn syrup showed good oil retention and water dispersibility (Klinkesorn et al. 2006).

24.4.1.4 Liposomes

Liposomes have been successfully used to deliver a range of oil-soluble bioactives. Banville et al. (2000) showed that incorporation of Vitamin D in cheese was improved with liposomal delivery compared to the use of a water-soluble preparation, but after long term storage (3–5 months) the stability of the liposome-encapsulated vitamin decreased. Multilamellar liposomes made from soy lecithin will incorporate β-carotene. Using a lecithin to β-carotene ratio of 1:0.05, efficiencies of up to 99.7% were obtained (Rhim et al. 2000).

24.4.1.5 Inclusion Complexes

Cyclodextrin has been used to encapsulate conjugated linoleic acid. Encapsulation of conjugated linoleic acid stabilized it against oxidation for 80 h at 35°C (Park et al. 2002). The formation of an inclusion complex between bixin (a carotenoid) and α-cyclodextrin improved the solubility of the carotenoid in water and protected it against degradation in the presence of oxygen and light (Lyng et al. 2005).

24.4.2 Encapsulated Delivery Systems for Water-Soluble Bioactives

Water-soluble bioactives may also be protected from their environment by entrapment within a matrix (e.g., gel) or the use of lipid coats. Most research on delivery of water-soluble bioactives has been on systems for enriching foods with water-soluble vitamins and minerals and more recently on the use of bioactive peptides in food. The delivery of bioavailable iron has been of particular interest because of the widespread problem of iron deficiency.

24.4.2.1 Lipid Coats

Hard fats are used to coat water-soluble bioactives. Release occurs by heating above the melting point of the fat or by mechanical rupture. Fat coatings have been used for protecting many water-soluble materials, which may otherwise be volatilized or damaged during thermal processing and to deliver materials such as ferrous sulfate, vitamins and other minerals. The peptides of casein hydrolysates encapsulated in lipospheres were shown to have reduced bitterness (Barbosa et al. 2004).

24.4.2.2 Liposomes

Ferrous sulphate encapsulated in soy lecithin liposomes has been used to deliver iron. These preparations have improved bioavailability compared to ferrous sulphate directly added in milk and dairy products (Boccio et al. 1997; Uicich et al. 1999). Albaldawi et al. (2005) reported that the addition of encapsulated haem iron in lecithin/cholesterol liposomes resulted in improved rheological properties of bread dough and the sensory properties of baked bread.

Liposomes, like single lipid coats, have been examined for their ability to deliver peptides and proteins. Encapsulation of casein peptides in liposomes (0.5–1 μm in size at a rate of 50%–60%) significantly reduced bitterness and increased stability (Morais et al. 2005). Hsieh et al. (2002) showed that α-amylase can be protected against the action of pepsin at low pH, demonstrating the protection that may be offered during transit through the stomach.

24.4.2.3 Protein Gels

Cold-set whey protein gels obtained by addition of calcium ions to preheated whey proteins have been used to deliver iron (Remondetto et al. 2002). By modulating the conditions of formation, gels with different microstructures (particulate or filamentous) were formed with different encapsulating properties. Filamentous whey protein gels were more efficient than particulate gels in delivering bioavailable iron to the intestine, as less iron was released at acidic but more at alkaline pH (Remondetto et al. 2004).

24.4.2.4 Multiple Emulsions

Vitamin B_1 was entrapped in an inner aqueous phase of a double emulsion stabilized by a mixture of whey protein isolate and xanthan as the external gum. The release of the vitamin was modulated by altering the pH or the ratio of the two biopolymers. The increased rate of release of the vitamin as pH was increased from 2 to 7 has been attributed to the decreased electrostatic interaction between whey protein isolate and xanthan. Increasing the rigidity of the external interface by increasing the amount of xanthan also decreased the rate of release of the vitamin (Benichou et al. 2004).

Immunoglobulins are prone to loss of their bioactivity through proteolysis and heating. The encapsulation of immunoglobulin Y obtained from egg yolk in a multiple oil-in water-in-oil emulsion was shown to be stable to pepsin and acid and more stable to heat treatment (95°C for 10 min) than the nonencapsulated form (Cho et al. 2005).

24.4.3 Encapsulated Delivery Systems for Probiotics

Probiotics have attracted interest because they have a role in the health status of individuals, particularly in improving gut health (Saarela et al. 2002). There are many strains of probiotic bacteria, with varying susceptibilities to acid, heat and oxygen.

There is much interest in ensuring that live bacteria, which are delivered through foods, survive during storage and through stomach acids.

24.4.3.1 Polysaccharide Gels

Probiotics have been entrapped in gels or beads made with various carbohydrates. Adhikari et al. (2000) examined the use of kappa-carrageeenan for encapsulating bifidobacteria for yoghurt applications. Microencapsualtion of *Bifidobacterium longum* B6 and *Bifidobacterium longum* ATCC 15708 improved the viability of the cells, but the taste of the yoghurt was different. Alginates have been used alone or in combination with other carbohydrates as encapsulating agents to improve survival of probiotics. A range of carbohydrate-based systems containing *Lactobacillus reuteri* was prepared using either phase separation or extrusion. Alginate and alginate/starch were found to provide superior protection to the bacteria in gastric fluid compared to those prepared from kappa-carrageenan with locust bean gum or xanthan with gellan (Muthukumarasamy et al. 2006).

Coating of alginate particles containing *Lactobacillus bulgaricus* KFRI 673 with chitosan improved their survival in simulated gastric and intestinal fluids as well as during storage (Lee et al. 2004). Co-encapsulation of prebiotics (e.g., HiMaize) with *Lactobacillus acidophilus* CSCC 2400 or CSCC 2409 in alginate improved the viability of the bacteria on exposure to low pH, bile salts and in yoghurt, although the improvement was dependent on the level and type of prebiotics used (Iyer and Kailasapathy 2005). However, encapsulation of *Bifidobacterium* spp. and *Lactobacillus acidophilus* in an alginate–starch matrix did not protect the bacteria against acid and bile conditions (Sultana et al. 2000). The different degree of protection afforded to microorganisms may be due to the different conditions used, the nature of the ingredients used or the different susceptibilities of different strains to environmental conditions.

24.4.3.2 Two-Phase Aqueous Delivery Systems

A novel all-aqueous-based system that relies on the thermodynamic incompatibility of biopolymers has been described for the encapsulation of probiotics. A two-phase system comprising of polyvinyl pyrolidone and dextran was used as the carrier material for a probiotic bacterium in the preparation of spray dried preparations of probiotics. The survival of the bacteria (*Enterococcus faecium* E74) was dependent on the composition of the system. Although the survival rate during drying was better when only dextran was used, there were better survival rates of the bacteria during storage with the two-phase carrier system (Millqvist-Fureby et al. 2000).

24.4.3.3 Emulsion Systems

Probiotics may be encapsulated in protein-based emulsions. Picot and Lacroix (2003) prepared microcapsules by emulsifying milkfat containing micronized skim milk powder (as a surrogate for freeze-dried bacteria) with heat denatured whey proteins and then spray drying. Incorporation rates of up to 58% milk fat and 29% skim milk

powder were obtained. Encapsulation of the bacteria (*Bifidobacterium infantis*) into a protein-resistant starch–oil emulsion with prebiotics prior to spray-drying improved the storage stability of the resultant probiotic powder during nonrefrigerated storage and protected the bacteria at gastric pH. The bacteria were released from the system on exposure of the microcapsule to simulated intestinal fluid (Crittenden et al. 2006).

24.5 Food as a Delivery System for Bioactives

Key trends influencing the development of the functional food and drinks markets include the increase in healthy eating, increased demand for natural and organic foods, snacking and convenience foods. Many more foods are now being used as delivery systems for bioactives including fruit juices and other beverages, dairy foods, cereal-based products, sports bars, confectionery, baked goods and spreads.

The demand for healthy foods has led to the development of several entirely new healthy ingredient and additive categories (Heasman and Mellentin 2001; Sloan 2004). An entirely new sector of functional food ingredients has developed, including products such as omega-3 fatty acids and phytosterols for cardiovascular health, prebiotics and probiotics for gut health, antioxidants, polyphenols and phytochemicals, and bioactive peptides. The established vitamins and minerals sector has also benefited from this functional food trend, with more and more products being fortified with beneficial vitamins and minerals.

Successful delivery of bioactives into foods requires that the bioactive is stable during food processing and throughout the food product's shelf-life and is in a bioavailable form when the food is consumed. The bioactive must be protected from possible interaction with other ingredients. The food matrix used as the delivery vehicle has to be considered. For incorporation of bioactives into dry powder food applications, the properties of the microencapsulated bioactive powder properties must match those of the final powder mix (e.g., the particle size, water activity, bulk density) to ensure homogeneity. When a bioactive is added to a processed food product, the stage of processing at which the bioactive is added can influence whether it is successfully incorporated (Augustin 2003; Sanguansri and Augustin 2006a) as can the formulation of the bioactive ingredient and its format. This means that the supplier of the bioactive (in both its raw or encapsulated form) has to work in conjunction with the food manufacturer as the formulation of the final food and the manufacturing processes influence the stability and bioavailability of the added bioactive.

There also needs to be evidence for the delivery of the bioactive to the desired site in the body to obtain the desired health outcome. This is important as the long-term success of functional foods depends on scientific evidence to substantiate valid health claims. As the knowledge about the physiological function of bioactives, the mechanism of their action, the effective doses and the desired site for release of bioactives grows, encapsulation will be increasingly used for targeted release of bioactives for improving the health of consumers (Chen et al. 2006).

24.6 Emerging Trends

The availability of a very limited range of suitable encapsulant materials allowed for food applications still remains the biggest challenge in material selection, especially when more sophisticated microcapsule properties are increasingly being required by the food manufacturers to deliver the types of fortified foods with the health benefits demanded by consumers. Not only are consumers interested in the effective delivery of traditionally recognized bioactives, but there is also increasing demand to maximize their health benefits by combining a number of bioactives into a single food. As this interest in the delivery of cocktails of bioactives for specific health outcomes grows, there will be increasing demands on the properties of encapsulated ingredients and the encapsulation technologies used to produce them.

The use of emerging food technologies such as ultrasound, static high-pressure processing and microfluidisation may provide the platform for the creation of novel microstructured assemblies to meet future needs and provide new processing or preprocessing capabilities for encapsulation systems.

Developments in nanotechnology and control over the self-assembly behavior of components is expected to provide new insights into delivery of bioactives (Mezzenga et al. 2005; Chen et al., 2006; Sanguansri and Augustin 2006b). The development of nanoparticles with different size and structure is expected to provide a new generation of encapsulated bioactives, (Moraru et al. 2003).

Real-time monitoring during consumption of foods, to ascertain the site and the rate of release of bioactives using nondestructive methods will be needed to help develop models to link structure to breakdown during gastrointestinal tract transit. The substantiation of health claims is central to the provision of evidence-based interventions based on food. Human-based trials will be needed to provide unequivocal evidence of bioactive performance.

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24.7 References

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