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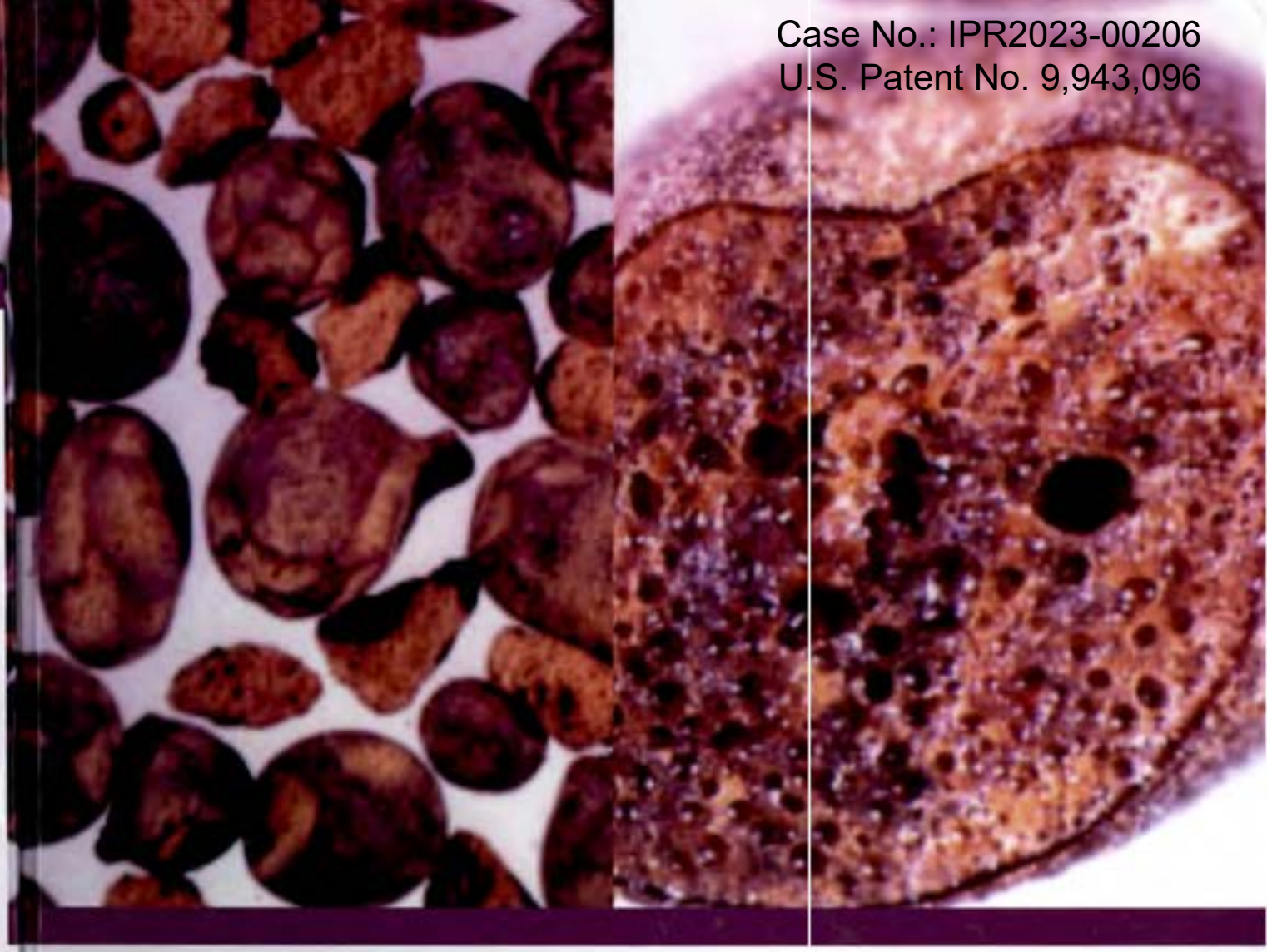

Microencapsulation in the Food Industry

A Practical Implementation Guide

Edited by
Anilkumar Gaonkar, Niraj Vasisht,
Atul Khare, and Robert Sobel

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Microencapsulation in the Food Industry

A Practical Implementation Guide

Edited by

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Microencapsulation in the Food Industry is written for those who see the potential benefit of using microencapsulation but need practical insight into using the technology. With coverage of the process technologies, materials, testing, regulatory and even economic insights, this book presents the key considerations for putting microencapsulation to work.

An **Introduction** to the field of microencapsulation as applicable to the food industry is the first of 7 sections presented. **Concept of Microencapsulation** describes how the concepts of mass transfer, heat transfer, kinetics, diffusion coefficient, partition coefficient, solubility, permeability, release etc. can be used in solving formulation development challenges. **Process Technologies in Microencapsulation** presents the different microencapsulation technologies along with relevant equipment design aspects, their critical process attributes and factors that impact the phase transition to create microcapsules and microspheres. The state-of-the-art for different processes are highlighted along with commercial examples and with relevant patent literature assisting the reader from an industrial scientist's perspective. **Materials Used in Microencapsulation** explains the matching of shell materials to the appropriate encapsulation technique, in addition to considering "best use" of selected carriers and techniques as they translate into final product form. **Testing And Quality Control** provides information on physical and chemical characterization methods for encapsulated actives and food products containing them. **Regulatory, Quality, Process Scale-Up, Packaging, and Economics** explores the influence of ingredient regulations and how they are disclosed to the consumer (e.g., product labeling). **Microencapsulation Applications** bridges the fundamental research in microencapsulation to the applied research aspect of this art.

Key Features:

- Focuses on applying understanding to real-world products including functional foods, flavor and oxidation stabilization, and color and taste masking
- Includes the latest in processes and methodologies
- Maximizes microencapsulation efforts by providing starting points for frequent use scenarios
- Encompasses over 50 years of combined industry and professional teaching experience

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Chapter 20

Gelatin and Other Proteins for Microencapsulation

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20.1 INTRODUCTION

As nutritious food ingredients, proteins, such as gelatin, soy protein, zein, pea protein, and so on, have many superior properties making them good and widely used microencapsulation shell materials in the food industry.

Emulsifying property is important for microencapsulation shell materials, especially for microencapsulation of oil-soluble ingredients, such as flavor oil, oil-soluble vitamins, and fish oil, and so on. Due to its amphiphilic nature, protein can orient at the oil–water interface with the hydrophilic groups oriented toward the water phase and the hydrophobic groups oriented toward the oil phase to lower the interfacial tension and form the emulsion. Protein can also build the protective barrier around the oil droplet to prevent the emulsion coalescence and maintain the stability of the formed emulsion (Zayas, 1997).

Through microencapsulation, the volatility, flavor, odor, and reactivity of food ingredients are suppressed. The protection of the microencapsulated contents from degradation or loss is provided by the barrier properties of the shell materials. Oxygen is involved in many degradation reactions in foods, such as fat and oil rancidity and vitamin loss. As a group, proteins appear to have lower oxygen permeability (Avena-Bustillos et al., 2011). Table 20.1 lists the oxygen permeabilities of different protein films.

Crosslinking can enhance the barrier properties of protein films, which can be achieved by either chemical or enzymatic treatment. Aldehydes, such as glutaraldehyde and formaldehyde, are generally used to chemically crosslink proteins. However, the toxicity of aldehydes enormously limits the exploitation of their use in food applications. To overcome the restriction in using toxic crosslinkers, microbial transglutaminase (MTGase) has been proposed as a crosslinking reagent for proteins. MTGase catalyzes an acyl-transfer reaction between the γ-carboxyamide group of peptide-bound glutamine residues (acyl donors) and a variety of primary amines (acyl acceptors), including the ε-amino group of lysine residues, to form an ε-(γ-glutamyl)lysine isopeptide bond (Motoki and Seguro, 1998). Crosslinking protein via Maillard reaction is also widely used in microencapsulation (Graham, 1996). The "Maillard crosslinks" can be formed by heating a protein in the presence of a reducing sugar. The Maillard reaction could also generate antioxidative compounds that can be useful for retarding the oxidation of core material (Amarowicz, 2009).

In addition to the emulsifying and barrier properties, most proteins have high water solubility, good drying property, and are widely available and generally regarded as safe (GRAS), making them ideal shell materials for microencapsulation in the food industry.

20.2 GELATIN

20.2.1 Gelatin Manufacture: From Collagen to Gelatin

As a substantially pure protein food ingredient, gelatin is obtained by partial hydrolysis of collagen derived from the skin, white connective tissue, and bones of animals (Haug and Draget, 2011).

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TABLE 20.1 Oxygen Permeability of Protein Films

Protein Type	Test Conditions	Oxygen Permeability, $cm^3\ mm\ m^{-2}\ d^{-1}\ kPa^{-1}$
Pork skin[a]	23°C, 55% RH	0.0055
Cattle hide[a]	23°C, 55% RH	0.0048
Warm water fish skin (catfish)[a]	23°C, 55% RH	0.0035
Cold water fish skin (Alaska pollock)[a]	23°C, 55% RH	0.0023
Soy protein isolate[b]	25°C, 50% RH	0.0518
Corn zein[c,d]	25°C, 0% RH	0.0120

[a]Avena-Bustillos et al. (2011).
[b]Cho et al. (2010).
[c]Krochta (2002).
[d]Glycerol used as plasticizer.

The primary structure of collagen consists of approximately 1050 amino acids called alpha chain. Collagen consists of three alpha chains and each alpha chain is coiled into a left-handed helix to form the secondary structure of collagen. The three chains are twisted around each other to form a right-handed triple helix, which is a rigid rod-like structure that makes the tertiary structure of collagen. These rods assemble together in a quarter-stagger arrangement to form the quaternary structure of the collagen (Schrieber and Gareis, 2007).

The triple-helical structure of collagen arises from its unique amino acid composition and sequence. Table 20.2 shows the amino acid composition of gelatin compared with globular protein (soy protein isolate). Glycine constitutes around 33% of the amino acid component, proline and hydroxyproline together about 22%. The amount varies among different species although the high content of proline and hydroxyproline seems to be characteristic of collagen (Boran and Regenstein, 2010). The predominant sequence of amino acids in alpha chain is Gly-X-Y, where X and Y could be any amino acids but are often proline and hydroxyproline, respectively. This unique sequence allows for the feasible helix formation. Proline and hydroxyproline display a lateral group that loops back to reattach the main chain and results in a ring, which limits the rotation of the polypeptide backbone and thus explains the stabilization of the collagen triple helix and gives collagen rigid structure to serve as the main stress-bearing component of tissues. The entire structure of the collagen is stabilized and reinforced by crosslinks in the form of covalent bonds (Schrieber and Gareis, 2007).

In the manufacture of gelatin, collagen containing raw material is first pretreated with acid or alkali to partially cleave the crosslinks, resulting in two types of gelatin. Type A gelatin is produced by the acid process, which is mainly used with pig skin and fish skin and are not heavily crosslinked (Schrieber and Gareis, 2007). In the acid process, the raw material is soaked in dilute mineral acid for up to 24 h. Type B gelatin is pretreated by the alkali process, in which the raw material is subject to alkali treatment. The alkali process is used for heavily cross-linked collagen, such as bovine hide, and takes longer time (Haug and Draget, 2011). During the pretreatment, the regular triple-helix structure of collagen is hydrolyzed to form random gelatin coils, which can then be extracted with hot water. After extraction, the gelatin solution is usually filtered, deionized, concentrated by evaporation, and then sterilized. The gelatin solution is then cooled to gel. The gels are extruded into "noodles" that are fed onto conveyor belts for drying. The dried gelatin noodles are then ground into particles of 0.1 to 10 mm in diameter, which is usually translucent, brittle, colorless or slightly yellow, nearly tasteless, and odorless (Haug and Draget, 2011).

Type A gelatin has an isoionic point of 7 to 9 based on the severity and duration of the acid treatment, which is hardly different from collagen. For type B gelatin, the alkali hydrolyzes the asparagine and glutamine side chains and almost completely converts them to aspartic and glutamic acid, respectively. As a result, type B gelatin has an isoionic point of 4.8 to 5.2 (Schrieber and Gareis, 2007).

Gelatin is generally regarded as safe (GRAS). Bovine spongiform encephalopathy (BSE) is virtually impossible to survive the gelatin production process (Haug and Draget, 2011).

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TABLE 20.2 Amino Acid Composition (% mole) of Gelatin and Globular Proteins

Amino Acid	Pork Skin[a]	Cattle Hide[b]	Warm Water Fish Skin (Catfish)[b]	Cold Water Fish Skin (Alaska Pollock)[c]	Soy Protein Isolate[d]
Aspartic acid	4.6	4.83	4.59	5.21	11.81
Hydroxyproline	9.1	10.39	7.72	5.3	N/A
Threonine	1.8	2.01	2.6	2.68	4.1
Serine	3.5	3.52	3.61	5.85	5.48
Glutamic acid	7.2	7.78	7.22	7.17	21.29
Proline	13.2	13.78	11.14	10.1	5.29
Glycine	33	28.74	34.01	35.7	3.86
Alanine	11.2	11.91	12.4	10.9	3.83
Valine	2.6	2.16	2.19	1.67	4.41
Cystine	0	0.04	0.1	0.14	0.06
Methionine	0.4	0.33	0.49	1.13	0.93
Isoleucine	1	1.28	1.17	1.07	4.48
Leucine	2.4	2.59	2.09	2.1	7
Tyrosine	0.3	0.22	0	0.24	3.71
Phenylalanine	1.4	1.33	1.3	1.2	5.3
Hydroxylysine	0.6	0.86	0.58	0.61	N/A
Lysine	2.7	2.77	3.1	2.78	5.39
Histidine	0.4	0.47	0.6	0.8	2.9
Arginine	4.9	4.98	5	5.18	7.57
Tryptophan	0	N/A	N/A	N/A	N/A
Homocystine	N/A	0.02	0.09	0.16	N/A

[a]*Zhou et al. (2006).*
[b]*Avena-Bustillos et al. (2006).*
[c]*Chiou et al. (2006).*
[d]*Tang et al. (2006).*

20.2.2 Gelation of Gelatin

Gelatin is insoluble in cold water and swells or hydrates when stirred into cold water. The swollen gelatin particles can hold water 5 to 10 times their weight. When the temperature is raised above 40°C, the swollen gelatin particles dissolve and form a colloid solution or sol, in which the gelatin molecules behave as random coils in water. When the sol temperature is lowered the sol-gel transition happens, during which the gelatin molecules partly revert to the triple-helix structure of collagen as some crosslinks are formed. This sol-gel transition is thermoreversible because the bonding energy in gelatin is relatively weak (Schrieber and Gareis, 2007).

Forming thermally reversible gels is the most remarkable characteristic of gelatin. Gelatin solution will start gelling when the temperature drops below a certain point, which is called gelling temperature. The formed gel will start melting when the temperature increases above a certain point, which is called the gel melting point, and is usually lower than human body temperature. This melt-in-the-mouth property makes gelatin superior as the shell material to encapsulate

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TABLE 20.3 Gelling and Melting Temperature of Gelatins

Gelatin Type	Gelling Temperature °C	Melting Temperature °C
Pork skin[a]	27	29
Cattle hide[b]	24.7	32.3
Warm water fish skin (catfish)[a]	22	25
Cold water fish skin (Alaska pollock)[c]	11.9	21.2

[a]Liu et al. (2008).
[b]Gudmundsson (2002).
[c]Zhou et al. (2006).

flavors and nutrients in the food industry (Baziwane and He, 2003). Table 20.3 shows the gelling and melting temperatures of different gelatins. In general, mammalian gelatin has the highest gelling temperature, followed by warm water fish gelatin; the cold water fish gelatin has the lowest gelling temperature.

Gel strength is the most important attribute of gelatin. It depends on the gelatin type, molecular size, concentration, temperature, and setting time. Gelation of gelatin is a slow setting process and the full gel strength is attained after 15 h. When determined by the standard method (GMIA, 1986), gel strength is graded by the Bloom value. Gelatin gel is made by cooling 6.67% gelatin solution at 10°C for 17 h. The gel strength, or Bloom value, is measured as the force, in grams, required to impress a 0.500 in diameter plunger to a depth of 4 mm into the surface of the gel. Commercial gelatins have Bloom values from 50 to 300 Bloom grams, varying from low Bloom (<150), medium Bloom (150–220), to high Bloom (>220) types. Gelatin is usually priced according to the gelling ability; consequently, the higher the Bloom, the higher the price.

Gelatin is a mixture of polymers with different chain lengths. The molecular weight distribution of gelatin is important for its gelation properties. For example, gelatin with high molecular weight normally has high gel strength and high gelling temperature. In the gelatin manufacturing process, with the control of pretreatment and extraction conditions, the desired molecular weight distribution of gelatin is obtained (Haug and Draget, 2011). For example, lower temperature extraction produces high molecular weight gelatin because less hydrolysis of the polypeptide backbone occurs.

Gelatin type is another important factor for gelation properties. Normally, fish gelatin from warmer waters has a similar gel strength to mammalian gelatins and they share similar hydroxyproline content. Fish gelatin from cold water species has low or no gel strength, which is due to its low hydroxyproline content (Table 20.2) (Karim and Bhat, 2009). But there are advantages to using low Bloom gelatin as a stabilizer of emulsions. The emulsion can be produced at or below room temperature, which allows the loading materials (e.g., fish oil) to avoid harsh conditions (e.g., high process temperatures), which can be detrimental to the stability of the loading substance.

As shown in Table 20.1, gelatin has better oxygen barrier properties than globular proteins because of its more linear structure, leading to higher cohesive energy density and lower free volume (Miller and Krochta, 1997).

20.2.3 Gelatin as Shell Material in Microencapsulation

Gelatin has been used as shell material to encapsulate different food ingredients through different technologies due to its superior physical and chemical properties.

20.2.3.1 Spray Drying

In the food industry, spray drying is the most commonly used microencapsulation method. Gelatin microcapsules prepared by spray drying are good carriers for many food ingredients due to the emulsifying and film-forming properties of gelatin.

Lipophilic food ingredients are normally emulsified before being encapsulated by spray drying. Compared with globular proteins, such as casein and whey protein, gelatin is a relatively weaker emulsifier (Dickinson and Lopez, 2001). But gelatin has very good film-forming properties during spray drying. Matsuno and Adachi (1993) interpreted the drying curve of different microencapsulation wall materials in terms of the ability to form a dense network. They suggested that the gelatin drying curve showed that the drying speed decreased rapidly at the early stage of drying,

which implied the rapid formation of a dense film and a good protection of core ingredient. Gelatin is thus considered as the most suitable for microencapsulation using a spray-drying process (Gharsallaoui et al., 2007). Liu et al. (2001) used gum arabic and maltodextrin to encapsulate ethyl butyrate by spray drying. The retention of ethyl butyrate was quite low. By adding 1% gelatin they found that the formation of crust on the surface of the droplets was improved and the retention of ethyl butyrate was appreciably increased.

Gelatin is normally used together with hydrocarbon compounds as shell materials in microencapsulation by spray drying. Shu et al. (2006) prepared lycopene microcapsules using spray drying with gelatin and sucrose as shell materials. Sucrose was used as a plasticizer, which was also believed to promote the formation of spherical and smooth-surfaced microcapsules (Ozgur and Mustafa, 2005). Bruschi et al. (2003) developed propolis ethanolic extraction powder encapsulated in gelatin by spray drying. When mannitol was added in the formulation, the dried powder had a very smooth and uniform surface, and spherical shape. Mannitol retains some water molecules linked to its own structure and could fill the empty space of the microparticles, preserving the hydration, avoiding depressions on the surface, and assuring a more uniform wall of the obtained microparticles (Bruschi et al., 2003).

20.2.3.2 Gelation

Gelatin has been used for microencapsulation based on its gelation property. Different approaches can be used to encapsulate food ingredients in gelatin gels. Gelatin gels show high permeability and dissolve under warm conditions, so the crosslinking of gelatin matrix is normally essential.

A simple way to use gelatin gel for encapsulation is to directly incorporate the core materials in the gelatiu solution and then induce the gelation and crosslink the gel.

In patent US 5153177 (Bower et al., 1992), a crosslinked gelatin bydrogel was prepared to encapsulate fat-soluble vitamins. The gelatin was dissolved in 60°C water together with a reducing sugar, like fructose, and a salt, like calcium acetate. The oil containing fat-soluble vitamin was mixed into the solution and the mixture was allowed to gel. The gelled slab was dried at ambient condition and then kept at 70°C for 6 h for Maillard reaction crosslinking. The crosslinked slab was kept insoluble in boiling water. US Patent 4670247 (Scialpi, 1987) revealed a process called powder catch to make water-insoluble beadlets to encapsulate fat-soluble vitamins. The first step was the emulsification of the fat-soluble vitamins in gelatin and reducing sugar solution. The emulsion was then sprayed into collecting powder, which converted droplets to particles. The gelatin gelled during this process. The collecting powder can be starch or modified starch, such as starch esters. The particles were then separated and dried with air stream. The dried beadlets were heated to high temperature (90–180°C) to induce a Maillard reaction to make insoluble beadlets. US Patent 6444227 (Leuenberger et al., 2002) reported a similar process to prepare beadlets but crosslinked the gelatin matrix by radiation or enzyme treatment. Patent WO 2005/089569 (Diguet et al., 2005) applied these beadlets containing polyunsaturated fatty acid in the extruded foods with excellent sensory properties.

Another way to encapsulate food ingredients in gelatin gel constitutes two steps. Gelatin microspheres are first prepared, which are then loaded with the food ingredients.

Gelation-induced phase separation is normally used to prepare the gelatin microspheres. Iwanaga et al. (2003) used the water-in-oil emulsion technique to prepare gelatin microspheres. Gelatin aqueous solution (10%) was preheated to 40°C and added drop-wise into olive oil under stirring to form the water-in-oil emulsion. The emulsion was cooled to induce the gelation of the gelatin droplets under stirring. The collected gelatin microspheres were crosslinked using glutaraldehyde and then freeze dried. Kong et al. (2011) prepared gelatin microspheres by the water-in-water emulsion technique. Gelatin was dissolved in water under stirring at 60°C to form a homogeneous solution, which was used as the dispersed phase. This gelatin solution was added drop-wise into polyethylene glycol (PEG) aqueous solution under agitation at 60°C. The PEG solution formed the continuous phase in this water-in-water emulsion system. Then the gelatine microspheres were solidified by rapidly cooling to 5°C. The gelatin microspheres were purified by multiple washing with chilled water. These microspheres were then crosslinked with glutaraldehyde and dried. Morita et al. (2001) prepared gelatin microspheres by freeze drying aqueous solutions containing gelatin and PEG. Phase separation between gelatin and PEG during freezing was assumed to be the mechanism of the formation of gelatin microspheres.

The prepared gelatin microspheres can be loaded with ingredients by diffusion. Dinarvand et al. (2005) loaded gelatin microspheres with lactic acid by immersing them in an aqueous solution of lactic acid. Yamamoto et al. (2001) prepared an aqueous solution of the protein to be loaded and dropped it onto the prepared dry gelatin microspheres. Iwanaga et al. (2003) discussed the *in vitro* release rate of insulin from gelatin microspheres prepared with various crosslinking densities and concluded that the release rate could be controlled by the density of the crosslinking.

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20.2.3.3 Coacervation

Microencapsulation by proteins can be obtained by the formation of an insoluble layer of protein around fine droplets of core material. Usually, the formation of the insoluble layer is achieved by simple or complex coacervation. The first approach is based on the addition of compounds with high affinity for water, such as alcohols. The second approach is based on the formation of a protein-rich layer around the core material, by interaction with oppositely charged polymers (Magdassi and Vinetsky, 1995). The gelatin crosslinking process is often used to fix the shell structure and the coacervates can be dried by different ways, such as spray drying and freeze drying.

Patent US 5035896 (Apfel et al., 1991) described a simple coacervation process using low melting point fish gelatin being soluble at 5 to 10°C. The coacervation of fish gelatin can be brought by lower alkyl alcohols such as ethanol and inorganic salts such as sodium sulfate. In the gelatin-based microencapsulation process, normally gelatin must be heated to approximately 40°C or above to dissolve the gelatin. This temperature may adversely affect heat labile core materials. The process of this invention is particularly useful for heat-sensitive core materials to be encapsulated in gelatin shell.

In a complex coacervation process, gelatin has been widely used as positively charged polymer. Patent US 7727629B2 (Yan, 2010) revealed a complex coacervation process to make multicore microcapsules. Type A gelatin with 275 of Bloom and isoelectric point of 9 was dissolved in 50°C deionized water. Fish oil was dispersed into the gelatin solution followed by homogenization to form oil-in-water emulsion. The emulsion was diluted with deionized water at 50°C. The sodium polyphosphate solution was then added into the emulsion with agitation. The pH was then adjusted to 4.5 with a 10% aqueous acetic acid solution. During pH adjustment and the cooling step that followed, coacervates formed from gelatin and polyphosphate coated onto the oil droplets to form primary microcapsules. After cooling, the primary microcapsules started to agglomerate to form lumps under agitation. After further cooling, polymers remaining in the solution further coated the lumps of primary microcapsules to form microcapsules with an outer shell and average size of 50 μm. Once the mixture was cooled to 5°C, gluteraldehyde was added to crosslink the shell. The mixture was then warmed to room temperature and stirred for 12 h. Finally, the microcapsule suspension was washed with water. The washed suspension was then spray dried to obtain a free-flowing powder. The powder particle size would be a little bigger than the size of microcapsules in the slurry and vary according to the drying conditions.

Gelatin has been used in complex coacervation with different polyanions. With acacia as polyanion, Junyaprasert et al. (2001) prepared gelatin acacia complex coacervates with vitamin A palmitate in corn oil as the core material. Silva et al. (2012) used bovine gelatin and pectin as wall material to encapsulate lycopene by a complex coacervation process.

The gelatin coacervation process can be controlled by pH, polymer concentration, and agitation (Yan, 2010). Nakagawa and Nagao (2012) used a freezing process to control the gelatin complex coacervation. An oil-in-water emulsion stabilized by gelatin–acacia solution was prepared, and the pH of the emulsion was adjusted with acetic acid. When emulsion pH was adjusted to 3 and 4, clear phase separations were confirmed. When emulsion pH was adjusted to 4.7, the emulsion was visibly stable at ambient temperature. By freezing the pH 4.7 emulsion, complex coacervation was confirmed. The membrane properties were found to be dependent on the cooling rate during freezing. However, freezing the emulsions with pH levels of 3.0 and 4.0 caused damage to the membrane due to ice crystal formation.

Surfactants play an important role in the gelatin complex coacervation process. Bhattacharyya and Argillier (2005) and Li et al. (2009) enhanced the shell structure of the gelatin–acacia complex coacervation microcapsules using cationic surfactants. Li et al. (2009) enhanced the shell structure of the gelatin–sodium carboxymethylcellulose complex coacervate by using sodium dodecyl sulfate (SDS). Magdassi and Vinetsky (1995) prepared gelatin microcapsules using type A gelatin and anionic surfactant SDS with soybean oil as the core material.

20.3 SOY PROTEIN

Soybean is a species of legume that contains 35 to 40% of proteins. Ten percent of soy proteins are water-soluble albumins and around 90% of soy proteins are globulins (Fukushima, 2004). Globulins are soluble in diluted salt solutions. β-Conglycinin and glycinin are the two major globulins in soy protein. β-Conglycinin is a trimeric protein with the sedimentation coefficient (SC) of 7S and the molecular weight (MW) of 70 kDa, whereas glycinin is a hexameric protein and has the SC of 11S and the MW of 350 kDa (Fukushima, 2004). The ratio between β-conglycinin and glycinin, which varies with the species and production process, has a significant effect on protein functionality (Swanson, 1990). For example, the higher hydrophobicity and more easily unfolded structure in β-conglycinin make its emulsifying ability much stronger than that of glycinin (Fukushima, 2004).

In the food industry, soybean attracts people's attention partly due to the nutritional value of soy proteins and other components in soybean, such as genistin, and partly due to the functional properties of soy proteins in food systems. Soy protein has superior functional properties, such as fat absorption, gelation, emulsification, film forming, and good barrier property, which make soy protein a good microencapsulation shell material. However, off-flavors (grassy, beany, bitter, astringent, and chalky) and allergen issue limit the application of soy proteins (Okubo et al., 1992). Soy protein isolate (SPI) is the most widely used soy protein product used for microencapsulation in the food industry. Aqueous alkaline extraction followed by isoelectric precipitation and salt extraction are commonly used for producing SPIs.

20.3.1 Spray Drying

Soy protein has been used in microencapsulation through different techniques. Spray drying is the commonly used one. Solubility is important for a microencapsulation shell material used in the spray-drying method. Protein solubility results from the hydrophilic groups on the surface of the protein interacting with water. The pH of the system is a major factor in determining solubility of a legume protein, as solubility decreases around the isoelectric point of the protein. In general, most legume proteins have very low solubility between pH 4 and 6, but outside this range, solubility increases dramatically (Boye et al., 2010). Soy protein possesses good solubility in water at pH higher than 9 (Fukushima, 2004). Soy protein has good drying and film-forming properties (Nesterenko et al., 2012) and good fat-absorbing property, which make it widely explored in microencapsulation of fat-soluble ingredients by spray drying.

With flavor compounds as core materials, Charve and Reineccius (2009) evaluated the microencapsulation performance of SPI, whey protein isolate (WPI), sodium caseinate, modified starch, and gum arabic by spray drying. The proteins effectively reduced the oxidation of the core material, which was pronounced in modified starch and gum arabic shells. WPI and SPI were good materials for the production of dried flavors based on volatile retention during spray drying. However, they performed poorly during storage as nonenzymatic browning occurred. Sodium caseinate performed poorly due to the low infeed solids limited by viscosity and its susceptibility to browning.

Casein hydrolysate has high biological activity for nutraceutical food formulation, but its application is limited by its intense bitter taste and high hygroscopicity. Ortiz et al. (2009) encapsulated casein hydrolysate by spray drying with SPI as wall material, which significantly attenuated the bitter taste of the casein hydrolysate. The bitter taste of casein hydrolysate is directly related to the existence of hydrophobic groups, which were probably less exposed due to hydrophobic interactions with the SPI in the encapsulating process. But the microcapsules had much higher hygroscopicity. Favaro-Trindade et al. (2010) used SPI and gelatin mixture as the shell and made the spray-dried casein hydrolysate microcapsules with lower hygroscopicity.

Rascón et al. (2011) evaluated the performance of SPI and gum arabic on encapsulating carotenoid by spray drying. When gum arabic was used as shell material, the microcapsules were stable at low water activities during storage. But if water activity (a_w) is above 0.318, the degradation rate of carotenoid increased substantially, and if a_w is above 0.743, microcapsules became unable to keep their structural integrity and led to the gradnal dissolution of the walls. The microcapsules with SPI as wall material were very stable at high water activities from 0.721 to 0.851.

Emulsification is critical for microencapsulation by spray drying. Soy proteins exhibit high emulsifying properties compared to other plant proteins (Zayas, 1997). Like other proteins, SPI is an ineffective emulsifier near the isoelectric point, where charges and solubility are minimal.

Kim et al. (1996) investigated the abilities of gum arabic, sodium caseinate, WPI, and SPI to form orange oil emulsion. Although SPI generated the largest orange oil emulsion droplets, SPI-emulsified orange oil droplets were found the most stable against creaming during 10 days of storage at room temperature, which could be due to SPI-emulsified orange oil droplets being surrounded by the thickest membrane structures. The spray-drying study (Kim and Morr, 1996) showed that SPI was the most effective wall material for retaining orange oil during spray drying.

Soy protein has been investigated through different modification processes to improve the emulsifying property. Protein hydrolysis results in peptides with smaller molecular size and less secondary structure than the original proteins, which give increased solubility and decreased viscosity. A strictly controlled hydrolysis usually improves the emulsifying property of the protein. Wu et al. (1998) prepared soy protein peptides by papain hydrolysis and obtained high emulsifying properties and protein solubility. Nesterenko et al. (2012) modified SPI by enzymatic hydrolysis and N-acylation. The oil-in-water emulsions prepared with native or modified SPI and hydrophobic α-tocopherol were spray dried to produce α-tocopherol microparticles. The results demonstrated that emulsions prepared with modified SPI had smaller droplet size and lower viscosity compared to those prepared with native proteins. The efficiency of oil retention decreased after protein hydrolysis from 79.7 to 38.9%, but the grafting of hydrophobic chain by acylation improved efficiency of α-tocopherol retention up to 94.8%. Molina et al. (2001) reported that the emulsifying ability index (EAI)

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reached the highest level after treatment under 400 to 600 MPa. Wang et al. (2008) suggested that unfolding of proteins and subsequent exposure of hydrophobic groups during the high pressure treatment improved the emulsifying property of SPI. But high pressure treatment resulted in a gradual and significant decrease in emulsion stability index (ESI).

20.3.2 Coacervation

SPI is amphoteric and has an isoelectric point of pH 4.5, similar to that of gelatin. Its potential to be used as a polycation in complex coacervation has been explored. Mendanha et al. (2009) encapsulated casein hydrolysate by SPI–pectin complex coacervation. The bitterness was significantly masked. Compared with the spray-dried casein hydrolysate microcapsules (Ortiz et al., 2009), the complex coacervates had much lower hygroscopicity. Nori et al. (2011) encapsulated propolis extract with SPI and pectin by the complex coacervation process to obtain it in powder form. The microencapsulation also preserved the phenolic and flavonoid compounds present in free propolis from oxidation. Gan et al. (2008) used SPI as shell material and prepared fish oil microcapsules by simple coacervation. Microbial transglutaminase (MTGase) and ribose were tried to crosslink the microcapsules. It was found that compared to the use of ribose, the use of MTGase to crosslink SPI did not satisfactorily yield sufficient protection to the fish oil.

20.3.3 Gelation

Similar to gelatine, soy protein has been used as microencapsulation shell materials based on its gelation property.

Heat denaturation is normally regarded as a prerequisite for gel formation of globular proteins (Chen and Subirade, 2009). In a stable soy protein solution, the surface of the soy protein micelles is predominantly occupied by the hydrophilic group. Heating protein dispersion causes protein molecular unfolding, in which functional groups (such as sulfhydryl groups or hydrophobic groups) become exposed and interact with each other through disulfide interchange reaction or hydrophobic bonding leading to protein aggregation (Zhang et al., 2012). When the protein concentration is high enough, aggregation leads to formation of a gel. At lower concentrations, aggregation leads to precipitation of the protein. For SPI, the minimal protein concentration for gelation is around 6.6% (Bikbov et al., 1979). Disulfide bond formation on heating makes the gelation process irreversible. In contrast, gelatin gels are stabilized mainly by hydrogen bonds and the gelation process is thermoreversible. The gelation and characteristics of soy protein gel depend on the protein species and concentration, pH, and ionic strength. Minerals, especially calcium, can serve as a salt bridge to enable polypeptide chains to approach one another, facilitate the protein aggregation, and therefore enhance the strength of the gel. In the case of β-conglycinin and glycinin, the larger numbers of sulfhydryl groups and their topology in glycinin make glycinin gel much harder and more turbid in comparison with β-conglycinin gel (Fukushima, 2004). Chen and Subirade (2009) prepared SPI microspheres using the gelation method and loaded the prepared microspheres with riboflavin.

Excluding heat-induced gelation, enzyme-induced soy protein gelation was also explored for microencapsulation purposes. Cho et al. (2003) encapsulated fish oil using the double emulsification and subsequent enzymatic gelation method. SPI was used as shell material and it showed higher reactivity with MTGase.

20.4 ZEIN PROTEIN

Commercial zein is essentially a by-product of the corn wet-milling industry. The protein content of corn varies from 6 to 12% on a dry basis. According to the solubility in selected solvents, corn proteins primarily include albumin (soluble in water), globulin (soluble in salt), glutelin (soluble in alkali), and zein (soluble in alcohol). Depending on the protocol used for protein fractionation, zein content ranges from 35 to 60% of the total protein of corn (Shukla and Cheryan, 2001). Zein contains a rich amount of glutamic acid (21–26%), leucine (20%), proline (10%), and alanine (10%), but lacks basic and acidic amino acids. The high amount of nonpolar amino acid residues and lack of basic and acid amino acids are responsible for the insolubility of zein (Shukla and Cheryan, 2001). Biologically, zein is a mixture of proteins with various molecular size, solubility, and charge. Esen (1986) separated zein into three fractions labeled α-zein, β-zein, and γ-zein, based on the solubility in isopropyl alcohol (IPA) solutions. α-Zein can be extracted using only aqueous alcohol, whereas the other zeins need a reducing agent in the solvent to be extracted. α-Zein accounts for 75 to 85% of the total zein. Commercial zein is mainly α-zein (Anderson and Lamsa, 2011) extracted by 86% aqueous IPA, which selectively dissolves α-zein and minimizes the other fractions of zein.

Zein has been employed as an edible coating for foods and pharmaceuticals because of its low moisture absorption, high thermal resistance, and good mechanical, oxygen, and aroma barrier properties (Shukla and Cheryan, 2001).

From a nutritional point of view, zein is deficient in essential amino acids, such as lysine and tryptophan. At the same time, its insolubility in water limits its use in food products. However, zein has good potential as a shell material in microencapsulation due to its unique hydrophobic properties. For example, it is interesting to use zein as a food grade biopolymer carrier of antimicrobials because zein is insoluble in conventional food beverages (Zhong and Jin, 2009).

20.4.1 Spray Drying

Zein can form tough, glossy, hydrophobic, greaseproof films that are resistant to microbial attack, with excellent flexibility and compressibility. Therefore, zein has been explored for microencapsulation through spray drying. Zhong and Jin (2009) prepared spray-dried zein microcapsules to encapsulate lysozyme, an antimicrobial. Sustained release of lysozyme was obtained, which is important for an antimicrobial to enhance its efficacy. Quispe-Condori et al. (2011) prepared flax oil microcapsules using zein as a coating material by spray and freeze drying. It was demonstrated that spray drying had much higher microencapsulation efficiency than freeze drying.

20.4.2 Zein Microspheres by Solvent Evaporation

Zein is soluble only in aqueous ethanol, so aqueous ethanol may be used as a solvent to dissolve both lipophilic bioactive compounds and the carrier zein. Ethanol is easily removed through evaporation, which makes it possible to prepare zein microspheres by phase separation in a nonsolvent followed by solvent removal by evaporation.

Parris et al. (2005) reported a process to make zein microspheres to encapsulate essential oils. In the process, the oil and corn zein were dissolved in aqueous alcohol, and then rapidly sheared in water containing a small amount of dispersant. The decrease of overall ethanol concentration resulted in the precipitation of oil-loaded zein particles. The suspension of particles was freeze dried. Zhong and Jin (2009) used a similar method and prepared fish oil-loaded zein microspheres. Su (2012) encapsulated citral flavor in zein by the solvent evaporation method. Patent WO 91/06286 prepared zein microspheres containing insulin. Zein was dissolved in 90% ethanol to form the encapsulating phase. Insulin was added to the zein solution. The mixture was dispersed into an immiscible phase (corn oil) with agitation. The first solvent (ethanol) is then removed by heating the mixture to 45°C, then cooled down to room temperature. Zein is insoluble in corn oil, and precipitates after evaporation of ethanol, forming a suspension of microspheres. The resulting microspheres were repeatedly washed with petroleum ether to remove the oil and filtered. The washed microspheres were then dried. Low temperature is one of the advantages of this process. Zein was also found promising in microencapsulation by other techniques, such as electrospinning (Torres-Giner et al., 2010).

20.5 PEA PROTEIN

Pea is a leguminous plant. Like other legume seeds, pea seeds contain high amounts of protein (20–30%). Pea proteins mainly exist as globulins (65–80%), which are also the main components in pea protein isolate (PPI) products. Globulins are composed of legumin 11S and vicilin 7S. Legumin is a hexameric oligomer consisting of 6 subunits with a molar mass of 60 kDa. Each subunit has one basic and one acidic subunit associated via a disulfide bond. Vicilin is a trimer and each subunit has a molar mass of about 50 kDa. The ratio of legumin to vicilin ranges from 0.2 to 1.5 depending on the genetics as well as the processing involved (Casey et al., 1982). The use of pea protein in the food industry for the formulation of new food products is very interesting because of its nonallergenic characteristic, despite the high nutritive value and good functional properties (Gharsallaoui et al., 2009).

20.5.1 Spray Drying

Pea protein has good film-forming properties (Viroben et al., 2000) and has been used as shell material in microencapsulation by the spray-drying method.

Gharsallaoui et al. (2012a) encapsulated medium chain triglyceride oil using PPI and maltodextrin by spray drying. They found that microcapsules with lower dextrose equivalent (DE) maltodextrin were shallow and presented rough surfaces, and microcapsules with higher DE maltodextrin were circular and uniform showing minimum cracks and dents on the surface. Qi (2004) encapsulated β-carotene in a PPI and corn syrup solid (DE40) system using emulsification and spray drying. They found that corn syrup solid had significant effect on the β-carotene retention during storage. Pea protein-stabilized emulsions can be very sensitive to environmental factors such as pH and ionic strength. For this reason, they are usually combined with another emulsifier/stabilizer such as high methoxyl pectin (Gharsallaoui et al., 2010).

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Gharsallaoui et al. (2012b) prepared multilayered emulsions using PPI and pectin to encapsulate flavor compounds (ethyl esters) and spray dried the emulsions to obtain dry powders. The results were compared with spray-dried powders from monolayered emulsion stabilized only by PPI. They suggested that the addition of pectin markedly improved the retention of the flavor compounds, which was possibly due to pectin being able to improve physical integrity of emulsion oil droplets during spray drying by preserving the β-sheet secondary structure of pea protein when pea globulins/pectin complexes are heated. Pea proteins were also successfully used for microencapsulation of hydrophilic ingredients by spray drying (Pereira et al., 2009).

To be used as a shell material in spray-dried emulsion, emulsifying property is important. Ducel et al. (2004a) reported that pea globulins were highly surface active at the oil–water interface, even more than gelatin at the same concentration. Karaca et al. (2011) compared emulsifying properties of PPI with SPI. PPI had lower emulsion capacity and stability, which was attributed to its higher surface hydrophobicity, lower surface charge, and lower solubility. They suggested that isolates produced by isoelectric precipitation had higher surface charge and solubility compared to those produced by salt extraction. Gharsallaoui et al. (2009, 2012a) evaluated the pH dependence of the stability of PPI emulsifying capacity. They reported the lowest emulsifying capacity at pH around the isoelectric point (pI $\approx$ 4.3), and both acidic and alkaline pH improved the emulsion capacity indicating the positive correlation between solubility and emulsifying capacity of PPI. The high emulsifying capacity of PPI at pH values below 4 and above 5 is due to intermolecular repulsion of charged amino and carboxyl groups, respectively; this aided dispersion of the protein molecules and increased repulsions between protein-stabilized oil droplets. At pH 4 to 5, the lack of electrostatic repulsions among emulsion particles promoted rapid coalescence and this decreased the volume of the emulsified fraction. They also stated that emulsions were more stable to creaming at acidic pH than at alkaline pH ranges, which was interpreted in terms of pea protein solubility, globulin dissociation, and oil-droplet surface electrostatic charge. Koyoro and Powers (1987) reported similar results. They found that at pH 4, pea protein globulins had minimum solubility and emulsifying capacity, which was improved when pH went higher or lower. They reported higher emulsifying capacity of pea protein globulins at pH 3 than pH 7, which followed their solubility profiles. As pea protein contains a large amount of reactive amino groups (lysine residue), chemical modification reactions onto the amino group, such as acetylation or succinylation, could effectively be carried out. These reactions were powerful to improve, for instance, emulsifying properties (De Graaf et al., 2001).

20.5.2 Coacervation and Gelation

Ducel et al. (2004b) demonstrated the potential of using PPI as polycation in complex coacervation to encapsulate medium chain triglyceride. Gum arabic was used as polyanion in the process and the optimum coacervation condition was obtained at pH 2.75 with a protein:polysaccharide ratio of 3:7.

PPI shows good potential as a matrix material for the microencapsulation of β-carotene, by means of supercritical CO_2 technology (De Graaf et al., 2001). In this process, microspheres based on PPI were first prepared by a water-in-oil emulsion technique. Stable PPI microspheres were obtained by gelation of the pea protein by heat treatment. The PPI microspheres were isolated and dried to give a free-flowing powder. β-Carotene was then loaded in the microspheres via supercritical CO_2 treatment.

20.6 CONCLUSION

Gelatin and other plant source proteins, such as soy protein, corn zein, and pea protein, have good emulsifying and oxygen barrier properties. Proteins, especially gelatin, have excellent drying property, film-forming property, and unique gelation property. Based on those physical and chemical properties, different techniques, including spray drying, coacervation, gelation, and so on, have been used to microencapsulate food ingredients with proteins as shell materials.

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