Chapter 8

Nanoencapsulation of Enzymes, Bioactive Peptides, and Biological Molecules

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8.1 INTRODUCTION

Throughout history, humanity has utilized natural resources in the pursuit for food and bioactive components for curing poisoning and also treatment of various diseases. The utilization of various bioactive metabolites, especially secondary metabolites are being directly attributed to the promotion of human health which further outlines the necessity to utilize such compounds in novel formulations such as functional foods. In the manufacture of industrial products, there is a clear need to deliver functional/bioactive components, including the food, pharmaceutical, cosmetic, and biomedical industries. These components represent a diverse range of substances, including proteins, enzymes, bioactive lipids, antimicrobials, antioxidants, nucleic acids, and medical drugs (Chen, Remondetto, Subirade, 2006; Chen, Weiss, & Shahidi, 2006; Shefer & Shefer, 2003; Ubbink, 2002; Ubbink & Kruger, 2006). In this chapter, we will primarily focus on the bioactive components and utilization of a variety of nanoencapsulation techniques relevant to these components with different methods.

Due to the advances in modern biological techniques, clinical symptoms of deficiency may be understood as a result of a long-term deficiency of micronutrients. Unfortunately, prior to such symptoms, the inadequate delivery of micronutrients may trigger the development of chronic diseases. At the same time, despite an optimum intake of micronutrients, some individuals still may exhibit a high risk for chronic diseases (Boushey, Beresford, Omenn, & Motulsky, 1995). All these findings highlight the importance of balanced diets and consumption of functional food products.

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Bioactive compounds consist of essential and nonessential natural compounds (e.g., vitamins or polyphenols), and they represent a small fraction of components in the food chain, but the critical point is that they most certainly were shown to demonstrate biological effect(s) on human health. In some cases, the presence of bioactive substances in food products provide health benefits that is well beyond the caloric value of the corresponding product.

The basis for the increasing interest in bioactive compounds is that knowledge from epidemiologic studies, such as the correlation between the consumption of certain compounds and occurrence risk of chronic diseases, has been increased. While until recently, vitamins and other micronutrients have been recommended only to avoid the classic symptoms of deficiency, current claims and lists of bioactive substances expanded significantly.

Bioactive compounds are expected to generate beneficial effects on human health even at considerably small amounts. The potential bioactivity and effectiveness of a certain bioactive material may be determined based on the monitoring of biomarker molecules *in vivo*. Incorporation of such compounds into our daily diet possibly decreases the risk of chronic diseases and improves our overall health conditions. For example, antioxidants found in plants are secondary metabolites serving several protective functions to the plants such as repelling insects (i.e., anthocyanins) and shielding against UV radiation, or regulating osmotic pressure, while exhibiting an astringent effect (Sepúlveda, Ascacio, Rodriguez-Herrera, Aguilera-Carbó, & Aguilar, 2011; Zarei, Azizi, & Bashiri-Sadr, 2010). Upon their consumption, bioactive effects in the human body proves advantageous such as inhibition of enzymatic and nonenzymatic oxidation reactions, binding of pro-oxidative substances, or scavenging free radicals.

For instance, antioxidants tend to stabilize and protect polyunsaturated fatty acids (PUFA) in food products due to their contribution to a few different stabilization mechanisms including reactions with free radicals, chelation of metals, and inhibiting the propagation of lipid oxidation. In the food products, antioxidant may form complexes with different juice components; they may induce a cloudy appearance or formation of a precipitate, especially, when complexed with proteins (Oziyci, Karhan, Tetik, & Turhan, 2013). Based on these findings, generation of bioactive bearing foods have to be carefully executed in order to optimize both sensory and biological quality. Due to the above mentioned advantages, antioxidants are among the most commonly investigated plant or fruit components (Bhandari, 2012; Fischer, Carle, & Kammerer, 2011; Gil et al., 2000; Jurenka, 2008; Viladomiu, Hontecillas, Lu, & Basseganya-Riera, 2013).

In this section, some of the most important types of bioactive compounds that need to be present in a balanced diet will be briefly discussed, along with a discussion of their physicochemical characteristics and the current challenges to their application in functional food formulations.

Among the priorities in the nanoencapsulation technologies, it is possible to mention the prevention of molecular degradation of bioactive compounds based on oxidation, hydrolysis, etc. Protection against the stresses that take place in processing, storage, transportation, and also during digestion are regarded as important. Consequently, potential exploitation of nanoencapsulation system in biomolecules is reviewed.

8.2 ENZYMES

Enzymes are a group of protein based molecules that catalyze biochemical reactions (Wiseman, 1987). In their molecular structure, some enyzmes also contain nonprotein components such as metal ions (i.e., copper, iron, magnesium, etc.) and vitamins. In our daily diets, we also consume a low concentration of enzymes both in processed and raw food products, whereas there have not been any clear findings on any damage that these enzymes caused to human up to date. Industrial utilization of enyzmes has been carried out since 1874, when Christian Hansen extracted rennin from calf stomach to manufacture cheese products (Nielsen et al., 1994). Since environmental conditions have a bearing on enzyme activity, their influence has to be accounted for.

8.2.1 Stability of Enzymes

There, generally, is a temperature range where each enzyme demonstrates its optimal performance. Unless enzymes were originally extracted from thermophilic organisms, this range mostly tends to lie between 30−50°C. In certain processes, such as the preparation of corn sirups, utilization of thermostable enzymes is necessary. Similarly, both for digestive and technological processes, there is an optimum pH range where enzymes demonstrate maximum stability and performance. While for pepsin the optimum pH range is between 1.5−3, trypsin is known to function most properly between pH 7−9. The changes in medium pH clearly affects the rate of enzymatic reactions. In that sense, encapsulation systems can stabilize the structural characteristics of enzymes and prolong their period of activity. In the ripening of cheese products, the two sets of major enzymes that are responsible tend to be endopeptidases and exopeptidases. When these enzymes were encapsulated in combination, ripening period and storage costs were found to be clearly reduced (e.g., Kınık, Kavas, & Yılmaz, 2003). Cheese products prepared with encapsulated enzymes were found to demonstrate enhanced taste, aroma, and textural approval compared to the control group samples (Peker & Arslan, 2011).

8.3 PEPTIDES

Peptides that are generated from food peptides under *in vitro* or *in vivo* conditions and that demonstrate biological functions and/or physiological effects are classified as bioactive peptides (Smacchi & Gobbetti, 2000). They are generally composed of short amino acid chains (i.e., 3−20 amino acids). Eggs, beans, fish, and corn proteins possibly constitute the most important sources of bioactive peptides along with dairy proteins (Ahn, Park, Atwal, Gibbs, & Lee, 2009; Majumder & Wu, 2009; Narva, 2004).

Although some sequences in proteins remain as inactive constituents, once hydrolyzed with digestive enzymes or during fermentative or other technological processes, they tend to demonstrate their bioactive properties (Korhonen & Pihlanto, 2006; Möller, Scholz-Ahrens, Roos, & Schrezenmeir, 2008; Roufik, Gauthier, & Turgeon, 2006; Schanbacher, Talhouk, & Murray, 1997; Schanbacher, Talhouk, Murray, Gherman, & Willett, 1998; Smacchi & Gobbetti, 2000). Based on their bioactivities, dietary intake of bioactive peptides may reduce the risk of chronic diseases and boost the immune system of the consumers. Consequently, a variety of peptides that demonstrate antimicrobial, antioxidative, antithrombotic, immune system regulating, and blood pressure lowering activities were found, and their bioactivities are possibly related to their amino acid composition and sequence (Korhonen & Pihlanto, 2006). Based on such bioactivities, functional food products can be manufactured (Park, Morimae, Matsumura, Nakamura, & Sato, 2008) and encapsulation systems may be utilized accordingly. Especially at reasonable cost levels, due to their safety, reliability, and high nutritional values, peptides and proteins may be utilized as functional food ingredients (Park et al., 2008).

8.4 PHYTOSTEROLS

Phytosterols are a group of phytochemicals that include compounds such as stigmasterol, β-sitosterol, and campesterol. Plant stanols, which are found naturally at lower concentrations than sterols, can be produced by the hydrogenation of phytosterols. Phytosterol concentrations in vegetable oils range from 0.1% to 1.0% (Chaiyasit, Elias, McClements, & Decker, 2007) and typical phytosterol consumption is in the range of 200 to 400 mg/day. The production of phytosterol fortified foods has become popular due to the ability of phytosterols to decrease total and low-density lipoprotein cholesterol in humans by inhibiting the absorption of dietary cholesterol (Wong, 2001; Ostlund, 2004).

Daily intake of 1.6 g phytosterols was found to lead to an approximately 10% reduction in LDL cholesterol (Hallikainen, Sarkkinen, & Uusitupa, 2000). The intestinal absorption of phytosterols is very low so dietary phytosterols do not have adverse effects on health. Phytosterol incorporation

into food formulations is quite challenging due to their high melting point and tendency to form insoluble crystals. However, PUFA esterified phytosterols demonstrate a higher solubility. Upon ingestion of phytosterols esters, lipases hydrolyze the fatty acid to produce free phytosterols. Phytosterols have mostly been added to high fat foods (e.g., margarine) where solublization and dispersion were relatively simple. Phytosterol introduction into water-based foods require them either being suspended or emulsified. Phytosterol oxidation products have been observed in model systems, oils, and food products (Bortolomeazzi, Cordaro, Pizzale, & Conte, 2003; Cercaci, Rodriguez-Estrada, Lercker, & Decker, 2007; Dutta, 1997; Lambelet et al., 2003; Soupas, Juntunen, Lampi, & Piironen, 2004). It is not clear whether oxidized phytosterols tend to lose their bioactive properties or demonstrate any toxic effects *in vivo* in a manner similar to oxidized cholesterol. As with other bioactive lipids that are susceptible to oxidative reactions, nanoencapsulation of phytosterols could potentially increase their oxidative stability and consequently render enhance their bioavailability.

8.5 NUCLEIC ACIDS

DNA is a critical biomolecule used in gene therapy (Putnam, 2006), diagnostics (Dharmadi & Gonzalez, 2004), nanorobotics (Yurke, Turberfield, Mills, Simmel, & Neumann, 2000), and molecular evolution (Chakrabarti, Breaker, Joyce, & Deamer, 1994; Tawfik & Griffiths, 1998; Ghadessy, Ong, & Holliger, 2001). A general problem in the effective and efficient utilization of DNA in these areas is to prevent its degradation, which can occur due to mechanical shear forces (Murphy, Cano, Fox, & Willson, 2006), chemical degradation by nucleases (Putnam, 2006), or other processing related variables (Zelikin et al., 2007). It is logical to concentrate and/or preserve DNA by a physical barrier (Zelikin et al., 2007) possibly based on nanoencapsulation techniques. There are a number of methods utilized in gene therapy to restrict DNA degradation, including complexation of DNA with polycations (Putnam, 2006), blockcopolymer micelles (Kataoka et al., 2001), cationic lipids, or liposomes (de Lima, Simoes, Pires, Faneca, & Düzgüneş, 2001). Furthermore, DNA molecules can be entrapped within gels (Goh et al., 2004), micellar (Csaba, Caamaño, Sánchez, Domínguez, & Alonso, 2005), or polymeric microparticles (Ando, Putnam, Pack, & Langer, 1999). In order to prepare transcriptionally active nucleic acid formulations, DNA may be encapsulated within liposomes (Edwards & Baeumner, 2007; Tsumoto, Nomura, Nakatani, & Yoshikawa, 2001), water-in-oil emulsions and polyelectrolyte capsules, fibers, or nanoparticles.

Among the priorities in the nanoencapsulation technologies, it is possible to mention the prevention of molecular degradation of bioactive compounds based on oxidation, hydrolysis, etc. Protection against the stresses that take place in processing, storage, transportation, and also during digestion are

regarded as important. In this chapter, potential exploitation of nanoencapsulation systems in biomolecules will be reviewed.

8.6 LIPID FORMULATION TECHNOLOGIES FOR NANOENCAPSULATION OF BIOLOGICAL MOLECULES

8.6.1 Nanoemulsions

Nanoemulsions (also known as miniemulsions or submicron emulsions) are nanoscale droplets of multiphase colloidal dispersions formed by dispersing at least one liquid phase in other immiscible liquid(s) by physical shear-induced rupturing mechanisms (Liu, Sun, Li, Liu, & Xu, 2006; Mason, Wilking, Meleson, Chang, & Graves, 2006; Meleson, Graves, & Mason, 2004; Russel, Saville, & Schowalter, 1989). Different size ranges have been reported in the literature for nanoemulsions, i.e., less than 100 nm (Guo et al., 2007; Porras, Solans, Gonzalez, & Gutierrez, 2008; Shakeel & Ramadan, 2010), 10−100 nm (Talegaonkar, Mustafa, Akhter, & Iqbal, 2010), 100−500 nm (Anton, Benoit, & Saulnier, 2008; Constantinides, Chaubal, & Shorr, 2008; Rossi & Leroux, 2007; Tadros, Izquierdo, Esquena, & Solans, 2004), and 100−600 nm (Solans et al., 2005). Physicochemical characteristics and biological fate of emulsion systems highly depend on the mean particle size characteristics; therefore, the definition of size range is not a trivial point. From a nanotechnological perspective, having size ranges of less than 100 nm and possessing different properties than ordinary emulsions could be considered, since nanoemulsions have some interesting physical properties that can be applied to distinguish them from conventional emulsion systems.

Nanoemulsions are appropriate systems for the delivery of poorly water-soluble food ingredients, such as fish oil and lipophilic vitamins, because of their ability to enhance the solubilization and the possibility of absorption in the gastrointestinal (GI) tract (Jafari, Fathi, & Mandala, 2015). After ingestion in the human body, droplets readily disperse in stomach to small droplets of nanoemulsion, which promotes the rapid release of the encapsulated bioactive throughout the GI tract (Talegaonkar et al., 2010).

Couvreur, Blanco-Prieto, Puisieux, Roques, and Fattal (1997) reviewed the possibilities of peptide and olipeptide delivery via multiple emulsion systems. Since the inner aqueous phase has the capacity to stabilize aqueous peptides, W/O/W (water-in-oil-in-water) emulsions represent an appropriate means of peptide delivery. Similarly, W/O/W nanoemulsions that were decorated with alginate and/or chitosan were successfully utilized in the delivery of insulin to rats orally (Li et al., 2013), and pronounced hypoglycemic activity was observed. Balcão et al. (2013) demonstrated that lactoferrin bearing W/O/W nanoemulsions demonstrated antimicrobial activity.

8.6.2 Nanoliposomes

Hydrophobic/hydrophilic interactions among lipid/lipid and lipid/water interfaces are responsible for the formation of liposomes. Liposomes are formed in single and bilayer arrangements. Lipo-soluble and water-soluble vitamins can be entrapped in these nanocarriers for maintaining their stability in different media.

Nanoliposomal systems have been commonly utilized in antimicrobial peptide encapsulation and also in the stabilization of enzymes in cheese manufacture. Liposomal encapsulation of antimicrobial peptides, including nisin, other bacteriocins, or bacteriocin-like compounds (Laridi et al., 2003; Teixeira, dos Santos, Silveira, & Brandelli, 2008; Were, Bruce, Davidson, & Weiss, 2003), have been investigated in detail in the literature. Since antimicrobial resistance of microorganisms is a major concern, enhancing the efficacy of antimicrobials through means of encapsulation could be a critical contribution to industrial food safety issues. Nisin or nisin plus EDTA bearing liposomes demonstrated pronounced inhibition towards *Listeria monocytogenes*. Coencapsulation of nisin and EDTA demonstrated maximum inhibition of *E. coli* O157:H7 (Taylor, Bruce, Weiss, & Davidson, 2008).

Liposomal encapsulation enhanced the antimicrobial potential of pediocin AcH against *L. monocytogenes* (Degnan, Buyong, & Luchansky, 1993). In addition to commonly used dairy, soy, or egg phospholipids, Imran et al. (2015) utilized marine phospholipids in the preparation of nisin bearing liposomes. Liposomal encapsulation could be also instrumental in the protection of proteins or peptides from thermal and/or pressure induced denaturation or degradation (da Silva Malheiros, Sant'Anna, Micheletto, da Silveira, & Brandelli, 2011, da Silva Malheiros, Sant'Anna, de Souza Barbosa, Brandelli, & de Melo Franco, 2012).

Liposomal bacteriocins from *Lactobacillus sakei* subsp. sakei 2a inhibited *L. monocytogenes* growth in both microbial media and intentionally contaminated goat milk (Malheiros, Cuccovia, & Franco, 2016). Not in all cases though the encapsulated peptides were more effective than free peptides. For example, *L. monocytogenes* was inhibited more significantly by free bacteriocin than liposomal P34 (da Silva Malheiros et al., 2011).

Liposomal encapsulation of enzymes in cheese manufacture has also been utilized for a considerably long period (Kirby, Brooker, & Law, 1987). Liposomal enzymes may enhance product texture, reduce storage time necessary for flavor formation and reduce the enzyme concentrations used in the process (Mozafari, 2006); thus, increasing the profitability in cheese manufacture.

8.6.3 Nanostructured Lipid Carriers

Since an increasing extent of commercial applications are anticipated in the delivery of proteins, oligonucleotides, and DNA, the importance of

encapsulation systems including emulsions and liposomes increase. In addition, other lipid based carriers have been reviewed. Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) could also be instrumental based on their biocompatible features (Martins, Sarmento, Ferreira, & Souto, 2007). Especially, hydrophobic proteins and peptides can be immediately stabilized in these systems without requiring an additional aqueous phase unlike W/O/W emulsions. Especially since 40% of new molecules manufactured as drugs have hydrophobic characteristics, the importance of SLN and NLC systems will increase over time, while toxicity concerns related to emulsifiers have to be keenly investigated (Martins et al., 2007).

8.7 NATURAL NANOCARRIERS FOR NANOENCAPSULATION OF BIOLOGICAL MOLECULES

8.7.1 Casein Nanocapsules

Cow's milk contains approximately 30−35 g of protein per liter. Casein accounts for about 80% of all bovine milk proteins and is coordinated in casein micelles. Casein micelles are successful nanovehicles that concentrate, stabilize, and transport essential nutrients, mainly calcium, phosphate, and protein, for the neonate (DeKruif & Holt, 2003). Therefore, it is a natural nanodelivery system that is common to a variety of mammals, although the composition and physicochemical characteristics vary between species.

The micelles are spherical colloids, 50−500 nm in diameter (150 nm in average) (Fox, 2003), formed by the presence of four major casein phosphoproteins: α_{s1}-casein (α_{s1}-CN), α_{s2}-CN, β-CN, and κ-CN (molar ratio 4:1:4:1, respectively) (DeKruif & Holt, 2003; Fox, 2003).

The molecular size range of casein proteins are approximately 19−25 kDa (Eigel et al., 1984), whereas more than half of their amino acid residues have polar side chains (Morimoto, 1970), which in turn promote intra- and intermolecular hydrogen bonding (Khadka & Haynie, 2012). All these critical properties render caseins a suitable nanodelivery system for food and pharmaceutical applications in addition to being an important source of minerals and essential amino acids (Brugman et al., 2004).

A number of studies have been carried out in order to encapsulate bioactive compounds in casein micelles, and a few brief examples are reviewed in this section.

The β-casein can self-associate to compose micelles that are able to dissolve lipophilic compounds but is not economical for food applications at this time. (Anema & De Kruif, 2013; Haratifar, Meckling, & Corredig, 2014; Pan, Zhong, & Baek, 2013; Yazdi et al., 2013). Casein micelles can be dissociated into nanoclusters by high-pressure homogenization (Orlien, Boserup, & Olsen, 2010) which was used for the incorporation of vitamin D2 in reassembled nanoparticles of sodium caseinate composed upon

depressurization (Semo, Kesselman, Danino, & Livney, 2007), which possibly can be further utilized for the encapsulation of peptides, proteins, and DNA molecules. Similarly, casein micelles can also be dissociated by calcium chelating agents such as citrate and ethylenediaminetetraacetate (Udabage, Mckinnon, & Augustin, 2000) or at alkaline conditions (McMahon, Du, McManus, & Larsen, 2009). These agents could represent an alternative strategy to reform casein micelles and casein based fractions upon which bioactive materials may be encapsulated. Ghasemi and Abbasi (2014) investigated the effects of alkaline pH and ultrasound treatment on the formation, structural attributes, entrapment efficiency, and protective properties of natural casein micelles. When medium pH was raised, size of casein micelles increased and the hydrophobic material encapsulation capabilities. Power intensity and duration of ultrasonic treatment clearly affected the entrapment efficiency of casein micelles as well.

Crosslinking of casein micelles with the enzyme transglutaminase cause the formation of stable nanoparticles that compose of a covalently linked casein network. Transglutaminase caused crosslinking of casein micelles formed intramicellar bonds, but the substructure of crosslinked casein micelles stayed identical to that of native casein micelles. The intramicellar crosslinking raised the stability of casein micelles (Huppertz & de Kruif, 2008).

Pan, Yu, Yao, and Shao (2007) formed polyelectrolyte complex micelles by mixed β-casein with lysozyme. The micelles were heated, which induced the gelation of lysozyme and the entrapment of β-casein in the gel, generating narrowly dispersed spherical nanoparticles. The synthesis of β-casein-lysozyme nanoparticles was primarily a combination of polyelectrolyte complex formation and heat caused gelation. These nanoparticles showed pH dependent hydrophobicity, which may be funtional in the encapsulation and release of relatively hydrophobic compounds (Pan et al., 2007).

In conclusion, casein micelles are potential nanovehicles for biomolecules. Casein micelles can be utilized for nanoencapsulation of hydrophobic biomolecule substances for potential enrichment of low-fat or nonfat food products.

8.7.2 Nanocrystal Nanocapsules

Nanocapsules are defined in the literature as mostly an oily or hydrophobic cavity surrounded by a thin polymer wall. A broad variety of polymer materials, such as poly(lactide-co-glycolide), poly-e caprolactone, and polyalkylcyanoacrylate, can be used for the preparation of nanocapsules (Skiba, Nemati, Puisieux, Duchêne, & Wouessidjewe, 1996). Nanocapsules are promising applications, since they are ideal for the encapsulation of lipophilic bioactives (Vauthier-Holtzscherer, Benabbou, Spenlehauer, Veillard, & Couvreur, 1991). On the other hand, nanocrystals are crystalline clusters of a few hundred to a

few thousand atoms with the corresponding mean sizes of a few nanometers (Parak et al., 2003).

Nanocrystals are particularly utilized for poorly water soluble molecules (Caban, Aytekin, Sahin, & Capan, 2014). Their contribution to bioavailability occurs by enhancing both solubility and bioadhesion to the intestinal wall (Caban et al., 2014). In their utilization in pharmaceutical applications, nanocrystals enhance dissolution velocity and saturation solubility, reproducibility and repeatability of oral absorption, the amount of bioavailable dose, and patient compliance by the reduction of number of oral units that need to be used (Müller, Jacobs, & Kayser, 2001; Rabinow, 2005). Nanocrystals are especially favorable formulations for poorly soluble nutraceuticals like coenzyme Q10, rutin, hesperidin, apigenin, etc. (Shegokar & Müller, 2010). The potential findings on nanocrystals render them promising for food applications (Gülseren & Corredig, 2013; Tzoumaki, Moschakis, & Biliaderis, 2011a, Tzoumaki, Moschakis, Kiosseoglou, & Biliaderis, 2011b; Tzoumaki, Moschakis, Scholten, & Biliaderis, 2013).

8.7.3 Cyclodextrin Nanocapsules

Cyclodextrins (CDs) are cyclic oligosaccharides, which are produced from starch via cyclodextrin glucanotransferase (CGTase) enzyme (Szetjli, 1998). These starch derivatives are known as cycloamyloses, cyclomaltoses, and Schardinger dextrins (Villiers, 1891; Eastburn & Tao, 1994). CDs are comprised of six (α-CD), seven (β-CD), eight (γ-CD), or more glucopyranose units linked by α-(1,4) bonds (Del Valle, 2004). They are nontoxic ingredients, are not absorbed in the upper gastrointestinal tract, and are completely metabolized by the colon microflora. β-CD has been on the GRAS list since 1998, as a flavor carrier and protectant, at a level of 2% in numerous food products (Szente & Szejtli, 2004).

The preparation of CDs is simpler and cheaper than most other methods of nanoencapsulation (Marques, 2010) and has the potential to provide the following benefits:

- Stabilization of light- or oxygen-sensitive substances.
- Modification of the chemical reactivity of guest molecules.
- Fixation of very volatile substances.
- Improvement of solubility of substances.
- Modification of liquid substances to powders.
- Protection against degradation of substances by microorganisms.
- Masking the bitter taste of certain compounds.
- Masking pigments or the color of substances.
- Catalytic activity of cyclodextrins with guest molecules.
- Controling the release of materials.
- Reducing material toxicity.

All of these advantages of cyclodextrins make them favorable for applications in analytical chemistry, agriculture, pharmaceutical, and food and cosmetic manufacturing areas (Astray, Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gándara, 2009; Cabral Marques, 1994; Hedges, Shieh, & Sikorski, 1995; Loftsson & Duchêne, 2007; Jackson & Lee, 1991; Marques, 2010; Singh, Sharma, & Banerjee, 2002). Cyclodextrins have sweet taste; so this reason make them suitable for masking the unwanted taste and odor (Marques, 2010). They are resistant to many rigorous food-processing methods such as freezing, thawing, and microwaving (Astray et al., 2009).

CDs can be utilized to solubilize and stabilize several biomedically-significant peptides and proteins, including growth hormones (Brewster, Hora, Simpkins, & Bodor, 1991; Charman, Mason, & Charman, 1993), interleukin-2 (Brewster et al., 1991), monoclonal antibody MN12 (Ressing et al., 1992), aspartame (Prankerd, Stone, Sloan, & Perrin, 1992), tumor necrosis factor (Hora, Rana, & Smith, 1992), albumin (Katakam & Banga, 1995), g-globulin (Katakam & Banga, 1995), lactate dehydrogenase (Izutsu, Yoshioka, & Kojima, 1995), etc. For example, α-CD raised the solubility of cyclosporin A, an immunosuppressive agent, in eyedrop form. α-CD provided the drug to penetrate into the cornea with the least local toxicity (Ichikawa, Kanai, & Yamazaki, 1995; Kanai et al., 1989).

Peptides and proteins cannot always be fully encapsulated into inclusion complexes of cyclodextrins (Prokai et al., 1996). Appropriate complex forming sidechains can bind cyclodextrins, resulting in modified solubility, stability, and/or membrane transport characteristics (Szejtli, 1994). Prokai et al. (1996) characterized interactions of amino acids with cyclodextrins by electrospray ionization mass spectrometry. They binded α-cyclodextrin with protonated tryptophan and β-cyclodextrin with human insulin. They concluded that electrospray ionization mass spectrometry is a powerful technique for the analysis of binding CDs with amino acids and peptides.

In conclusion, cyclodextrins are very useful agents for nanoencapsulation of biomolecules, and their further exploitation is anticipated in biomolecule delivery in functional foods.

8.8 EQUIPMENT BASED TECHNOLOGIES FOR NANOENCAPSULATION OF BIOLOGICAL MOLECULES

Electrospinning and electrospraying are electrohydrodynamic processes. In these processes, a polymer solution can be spun or sprayed by the application of a high potential electric field to obtain fibers or particles, respectively (Faridi Esfanjani & Jafari, 2016; Katouzian & Jafari 2016). Electrospinning and electrospraying are used to produce new materials including encapsulation systems. Electrically charged jet of polymer solutions are used in electrohydynamic processes because of the producing fibers or particles at micron, submicron, or nanoscales. When compared to the other encapsulation methods, these processes are relatively easier, cheaper, and flexible and

present many advantages, while their utilization in the field of food processing is novel.

Electrohydrodynamic encapsulation methods comprise of four main components: (i) A high voltage source (1−30 kV) usually activated in direct current mode, though alternating current mode is also possible (Kessick, Fenn, & Tepper, 2004), (ii) a blunt ended stainless steel needle or capillary, (iii) a syringe pump, and (iv) a grounded collector either flat plate or rotating drum (Bhushani & Anandharamakrishnan, 2014). Electrospinning and electrospraying are accepted as "similar" technologies; however, there are several discrepancies which distinguish the two electrohydrodynamic processes.

In electrospinning, when electrical forces suppress the forces of surface tension in the charged polymer liquid, a charged jet excluded from the tip of a capillary tube elongates, which is known as Taylor cone, and goes towards a grounded surface. The solvent in the jet is vaporized during the flight, inducing mat of nanofibers deposited on the surface. Electrospun fibers are continuous and can vary in diameter of several nanometers to micrometers. These fibers can be produced by changing the collector or the electrical field (Chakraborty, Liao, Adler, & Leong, 2009). Polymer types, molecular weight, and concentration are critical factors for electrospinning because the feasibility of polymer electrospinning is determined by these factors (Chakraborty et al., 2009). For example, elastin-like polypeptides have been used as polymer materials in electrospinning methods. Electrospining is a simple and rapid encapsulation technique for the stabilization of bioactive compounds (Bhushani & Anandharamakrishnan, 2014; Brandenberger, Nüssli, Piech, & Widmer, 1999).

On the other hand, electrospraying is a process of liquid atomization by electrical forces. The difference between electrospinning and electrospraying techniques is the concentration of the polymer solution. When the solution concentration is high, the jet from Taylor cone is stabilized, and elongation takes place by whipping instability mechanism. If the solution concentration is low, the jet is destabilized due to varicose waves on the surface of the jet, leading to the formation of small and highly charged liquid droplets and, hence, fine droplets are formed. These highly charged droplets are self-dispersing in space, thereby preventing droplet agglomeration and coagulation (Brandenberger et al., 1999; Jaworek & Sobczyk, 2008).

Consequently, electrospinning and electrospraying techniques are commonly used for encapsulation of bioactive molecules; since they provide efficient means of bioactive encapsulation and enzyme immobilization, these techniques are also suitable for various food coating and development of novel filtration materials and active food packaging applications.

8.8.1 Enzyme Immobilization Based on Electrohydrodynamic Processes

Enzyme immobilization is a widely utilized method for most large-scale (i.e., industrial) applications since catalyst recycling, continuous operation, and

product purification become more controllable and reproducible with this approach (Li, Chen, & Wu, 2007). Electrohydrodynamic processes are quite instrumental in the encapsulation of enzymes since they are nonthermal process, which in turn protect the native structure of enzymes. When enzymes were immobilized onto several insoluble or solid materials, their thermal and operational stabilities were found to increase significantly (Mozhaev, Melik-Nubarov, Sergeeva, Šikšnis, & Martinek, 1990). Due to the reduced biocatalytic efficiency in many immobilized enzyme investigations, industrial applicability is generally low. Biocatalytic efficiency of enzymes can be enhanced though by the tailoring of the structures formed by carrier materials (Shinkai, Honda, & Kobayashi, 1991). Although nonporous materials severely limit the diffusion of enzymes, their capabilities of enzyme loading are quite limited as well. While, porous materials can provide high enzyme loading, their corresponding release rater are significantly faster (Hayashi et al., 1993). Based on these findings, nanofibers were utilized as the supports for enzyme immobilization with respect to both enzyme loading and enzymatic activity.

For example, Li et al. (2007) produced *Candida rugosa* lipase bearing polyacrylonitrile nanofibers by electrospinning. The immobilization was based on the amidination of polyacrylonitrile nanofibers. Enzyme molecules were determined to be covalently attached to the support and produced aggregates on the fiber surface, which also became more hydrophilic and resistant after enzyme immobilization. In another application, an amperometric biosensor was developed by electrospining poly(vinyl alcohol) (PVA) in the presence of glucose oxidase (Ren et al., 2006). The immobilized glucose oxidase was placed on the surface of Au electrode. The immobilized enzyme remained active inside the electrospun PVA fibrous membranes. The enzyme immobilization within PVA membranes demonstrated by the infrared (IR) and UV−Vis spectra and the scanning electronic microscope (SEM) micrographs while enzyme was shown to remain active. The biosensors displayed a rapid response time (1 s) and a higher output current (μA level) to glucose in the normal and diabetic level (Ren et al., 2006). PVA fibrous membranes were used in enzyme immobilization due to their high specific surface area and porous structure.

8.8.2 Nucleic Acid Encapsulation Based on Electrohydrodynamic Processes

As an alternative and promising technology, electrohydrodynamic processes are used for DNA encapsulation. DNA molecules were observed to lose their bioactivity and degrade within a few days following their conjugation to electrospun fiber surfaces and slowly being released into the nearby tissue would significantly preserve their functionality (Zamani, Prabhakaran, & Ramakrishna, 2013). While the particle core encapsulated bioactive molecules, the shell can be incorporated with bioactive molecules like nonviral gene-delivery vectors, which can act as a nonviral delivery vector for encapsulated DNA upon release. In another study, improved a coaxial electrospray

process to produce oligodeoxynucleotide encapsulated lipoplex nanoparticles for gene delivery. Incorporation of oligodeoxynucleotide and lipoplex nanoparticles is used in inhalation therapy. Compared to emulsion technologies, this process facilitated better controllability.

8.8.3 Protein Encapsulation Based on Electrohydrodynamic Processes

Native structure of proteins is formed by a one-dimensional linear chain of amino acids that has folded into a complex three-dimensional structure. In most cases, a "folded" protein will show various levels of structural organization. The structural and functional properties of peptides and proteins, the biocompatibility of these molecules, the nutritional necessity for protein in animals, and the tissue toxicity of several synthetic organic biopolymers, including those confirmed by the US Food and Drug Administration (FDA), have together encouraged interest in developing biomaterials made of proteins and polypeptides (Khadka & Haynie, 2012). Proteins demonstrate a wide range of functions, including the processes of enzymatic catalysis, cellular signaling, immune responses, cellular adhesion, and cellular cycle. Some other proteins are organized as structural fibrous materials. Protein fibers can be considered as building blocks of organisms, enabling scaffolding, stabilization, protection, elasticity, and motility at length scales ranging from nanometers to meters. Structural and functional attributes of fibrous proteins are increasingly being exploited to enhance the performance of synthetic biomaterials.

Electrospinning was studied to structure proteins on a nanometer length scale in a specific way (Nieuwland et al., 2013). Proteins are very difficult to electrospin, principally, because of their complex secondary and tertiary structures. Firstly, in order to achieve a spinnable system, protein molecules should be well dispersed in a random spiral conformation. Globular proteins have little interaction with each other that could facilitate entanglements during the spinning process. Caseinates, as a group of protein with a random spiral structure in water (i.e., no well-defined tertiary structure), may appear spinnable at the first glance. However, spinning pure caseinate from an aqueous dispersion was not possible. The inability to spin caseinate was most probably provoked by the aggregation of the caseinate molecules (Pitkowski, Durand, & Nicolai, 2008).

Proteins can be electrospun under conditions where they dissolve well (Bhardwaj & Kundu, 2010) in a random spiral conformation. When proteins are dissolved in solvents with high solvent qualities, it could be possible to distinguish between both intra- and intermolecular interactions between proteins and solubilize the resulting unstructured protein. This strategy was used for collagen and gelatin, where they were spun from hexafluoro-2-propanol (HFP), trifluoroethanol (TFE), or aqueous acid (with at least 40% acetic acid or formic acid) (Boland et al., 2004; Chen, Li, Li, & Guo, 2007; Chen,

Mo, & Qing, 2007; Huang, Gao, et al., 2004; Huang, Zhang, Ramakrishna, & Lim, 2004; Ki et al., 2005; Li et al., 2005; Matthews, Wnek, Simpson, & Bowlin, 2002; Songchotikunpan, Tattiyakul, & Supaphol, 2008). Silk and fibrinogen were spun from HFP or 98% formic acid, (Kawahara, Nakayama, Matsumura, Yoshioka, & Tsuji, 2008; Min et al., 2004; Wnek, Carr, Simpson, & Bowlin, 2003) and various globular proteins (among others hemoglobin and BSA) were spun from TFE (Barnes et al., 2006; Dror et al., 2008). Although these harsh solvents are usable for biomedical applications, their food applications are not permitted (Nieuwland et al., 2013).

Stephansen, Chronakis, and Jessen (2014) electrospun cod sarcoplasmic proteins. They determined that fish sarcoplasmic protein (FSP) fibers were insoluble in water and FSP concentration influenced the morphology of the electrospun FSP fibers. Aceituno-Medina, Lopez-Rubio, Mendoza, and Lagaron (2013) electrospun amaranth protein isolate in the presence of formic acid solution. They determined the effect of pH, type of solvent, and surfactant addition on the spinnability, morphology, and molecular organization of electrospun fibers. In their study, hexafluoro-2-propanol was used as a solvent for electrospinning process. In another study, whey protein isolate (WPI) and beta-lactoglobulin molecules were electrospun with poly(ethylene oxide) (PEO). It was determined that WPI/PEO composite nanofibers maintained their fibrous morphology at temperatures as high as 100°C, which is well-above the melting point of PEO (i.e., 60°C).

In conclusion, electrospinning and electrospraying methods can accelerate the expansion of food processing sector in encapsulation applications, food packaging, and edible coating. Electrospun nanofibers and electrosprayed nanoparticles present structural and functional advantages. The encapsulation of food bioactive compounds in electrospun fibers and electrosprayed particles contributes significantly to their stability and controlled release characteristics.

8.9 BIOPOLYMER BASED TECHNOLOGIES FOR NANOENCAPSULATION OF BIOLOGICAL MOLECULES

8.9.1 Protein Nanogels

Ovalbumin and lysozyme, which are hen egg proteins, can be utilized in order to manufacture nanogels. Once these proteins are thermally denatured, they are bonded by hydrophobic interactions, hydrogen bonds, and disulfide linkages. To stabilize surface structure of nanogels, electrostatic repulsive forces can be effectively tailored. Alkali reagents have been used in nanogel formation process for rendering protein nanogels edible and nutritional (Yu et al., 2006). It is possible to manufacture protein nanogels from food proteins (e.g., Chen, Lin, Sun, & Zhao, 2014), which in turn can be utilized in the encapsulation of bioactive compounds. Polypeptide nanogels could also

be utilized in the delivery of bioactives (Kim, Han, Kweon, Park, & Lim, 2013; Kim, Park, Kim, & Lim, 2013; Kim et al., 2013).

8.9.2 Chitosan-Based Nanogels

Chitosan is a widely utilized biopolymer in the delivery of bioactive compounds, especially macromolecules, due to its both physicochemical and biological properties (Kato, Onishi, & Machida, 2003). Because chitosan is water-soluble (i.e., in dilute acetic acid solutions, etc.) and positively charged, it can easily interact with negatively charged polymers or polyanions in aqueous environments. Furthermore, chitosan has been utilized as an absorption enhancer through the mucosal barrier in a variety of studies. In addition, chitosan based nanogels display low toxicity and good biocompatibility (Shutava & Lvov, 2006; Knapczyk et al., 1989). Consequently, they are also appropriate systems for the delivery of bioactive molecules such as proteins, peptides, and DNA. For example, peptide conjugation to chitosan nanogels was shown to successfully facilitate peptide delivery, while the gel structure remained unaffected by the inclusion of a bioactive (i.e., cardiac) peptide (Reis et al., 2012).

8.9.3 Alginate-Based Nanogels

Alginate-based nanogels represents nanogels formed by nano particles within a range of 250−850 nm with sodium alginate solution, calcium chloride, and poly-lysine. The interest for these nanogels is increasing in many fields such as antitubercular and antifungal drugs, therapeutic agents, and even gene delivery. Pescosolido et al. (2010) showed that, in combination with other polysaccharides, alginate is an appropriate raw material in protein delivery and release.

Alternative technologies based on poly(vinyl alcohol), poly(ethylene oxide), poly (ethyleneimine), poly(vinylpyrrolidone), poly-N-isopropylacrylamide, and hyaluronic acid nanogels are also utilized in the delivery of bioactive compounds and medicinal drugs.

8.9.4 Nanotubes

Nanotubes were discovered by Sumio Iijima, who is a Japanese electron microscopist, in 1991. Nanotubes demonstrate a characteristic hexagonal shape similar to the structure of an empty carbon tube, with the slight difference of having additional atom groups on two sides (Scott, 2005). They display thermal resistance at elevated temperatures and have a solid and flexible structure that might be used in many areas, including food industries, nanomedicine, and medical devices, etc. As an example relevant to food applications, it is noteworthy that when milk protein α-lactalbumin (α-La)

was hydrolyzed partially, it transformed into self-assembled nanotubes under suitable conditions (Graveland-Bikker & De Kruif, 2006, Graveland-Bikker, Fritz, Glatter, & de Kruif, 2006).

Recently, researchers indicated that in addition to α-La, some globular whey proteins, such as β-lactoglobulin (β-lg) and bovine serum albumin (BSA), also self-assembled into fibrillar structures at elevated temperatures and low pH values. These nanofibrils had a characteristic diameter of approximately 5 nm and range up to 15 μm in length. Protease enzymes from *Bacillus licheniformis* were found to partially hydrolyze α-lactalbumin molecules, and immediately afterwards hydrolyzed protein was exposed to calcium ions. Consequently, the formation of linear nanotubes was observed (Graveland-Bikker & de Kruif, 2006).

All of the major physicochemical characteristics of α-lactalbumin nanotubes render this component a promising encapsulation agent (Gouin, 2004). Firstly, since α-lactalbumin is a milk protein, its utilization in food industry is already quite common (Rajagopal & Schneider, 2004). Much similar to the casein micelle, which is a promising natural nanovehicle for delivering hydrophobic bioactives including probiotics, self-assembled α-lactalbumin nanotubes could play similar roles (Augustin & Hemar, 2009). Similar to casein micelles, in addition to the potential delivery of bioactives, protein nanotubes, as a compact system, concentrate protein molecules and, thus, facilitate their delivery.

There are also a variety of investigations in the literature where the encapsulation of proteins, peptides, and DNA molecules were carried out by carbon, lipid, and protein nanotubes (Davis et al., 1998; de la Escosura, Janssen, Schenning, Nolte & Cornelissen, 2010; Kameta et al., 2007; Kang et al., 2009).

8.9.5 Starch Nanoparticles

Starch, which is a storage carbohydrate in plants, is mostly presented in vegetables (30−50 g/100 g), cereal grains (40−90 g/100 g), immature fruits (40−70 g/100 g), and tubers (65−85 g/100 g). Starch is structurally made of amylose and amylopectin chains. While amylose is a linear chain that contains D-glucose units gathered by glycosidic α-1,4 bonds; amylopectin, which is a branched structure, consists of D-glucose units joined by α-1,4 and α-1,6 bonds (Gonçalves, Noreña, da Silveira & Brandelli, 2014). In addition to its critical importance in baked goods, native and modified starch products have been widely used in many areas such as fat replacers, excipients for tableting, drug delivery matrix formers, and food emulsifiers (Mahkam, 2010). Due to its biodegradable and biocompatible properties, starch is a promising, versatile, and inexpensive polysaccharide for drug delivery applications (El-Hag & Al Arifi, 2011; Chen, Li, et al., 2007; Chen, Mo, et al., 2007). In a variety of investigations, starch nanoparticles (SNPs)

were utilized and their size range is approximately between 10−1000 nm (Chin, MohdYazid & Pang, 2014). Utilization of modified starch in the manufacture of nanosized starch particles is practical, since the crystallinity, hydrophobicity and stability characteristics against enzymatic and thermal degradation need to be improved (Xu et al., 2010). SNPs have distinct advantages such as lower viscosity and small particle size, while considerable amounts of active ingredients can be loaded in these systems (Chen, Remondetto, et al., 2006; Chen, Weiss, et al., 2006).

When starch is used as an encapsulation agent, its crystalline arrangement is vital due to the possibility to produce more linkages among the polymer chains and generation of a wall material with improved characteristics such as reduced diffusion rate is necessary (Palma-Rodriguez et al., 2012). Crosslinking method is also widely used in order to enhance the functional properties of starch products. When the number of crosslinks in starch granules increase, the viscosity, water absorption capacity, and textural characteristics of starch suspensions will be improved. Based on these advantages, SNPs display a desirable elevated viscosity and optimum extent of water holding (Kramer, 2009).

Winarti, Sunarti, Mangunwidjaja, and Richana (2014) discussed the potential of SNPs in the encapsulation of bioactives. Both in the research related to SNPs and starch spherulites (Kong & Ziegler, 2014; Ma, 2010), there is an increasing amount of information in the area of bioactive molecule encapsulation, especially that of small molecules. In the following years, we anticipate a significant extent of progress in this area.

8.10 RELEASE OF PEPTIDES FROM NANOSCALE DELIVERY SYSTEMS

In order to establish peptide nanoencapsulation systems with the capabilities of slow-release of peptides, mostly a particle size range of 50−100 nm is utilized due to the prolonged stability of peptides (Couvreur & Puisieux, 1993). Environmental factors deeply affect the stability of encapsulated peptides, and an appropriate encapsulation system has to be used to prevent inactivation.

Also, since the major characteristics of proteins may be altered during metabolic activities, bioactive material (i.e., peptide) release needs to be tailored via the design of successful delivery systems. Immunoglobulins, serum albumin, lactoferrin, lactoperoxidase, and proteose-peptone are among the physiologically active milk serum proteins. During their enzymatic degradation, a variety of bioactive peptides, such as lactoferricin, alfa-lactophorin, serophorin, lactokinin, beta-lactophorin, beta-lactotensin, and albutensin, were shown to be formed (Cabuka et al., 2014). The correlation between the breakdown of nanoparticles and the extent of enyzme released from these systems was investigated (Martins et al., 1996). In addition to the importance of the

extent of release, the interactions with the nanoparticle matrices have a bearing on the activity of incorporated enzymes (Martins et al., 1996).

Rapid (i.e., burst) release was related to the loading of proteins on the particle surface (i.e., surface adsorption) rather than the incorporation within particle interior (Giovagnoli et al., 2005). Consequently, the design of delivery systems are quite critical. For example, lipid matrices are clearly good encapsulation systems for lipophilic bioactives rather than hydrophilic proteins (Reithmeier et al., 2001). However, for lipophilic peptides as well, it is possible to utilize SLN systems. In the case of leuprolide in prostate cancer treatment, encapsulation in SLNs and *in vitro* release characteristics were clarified by Gallarate et al. (2011). Relatively large peptides (13−43 amino acids) or recombinant human growth hormones were rapidly released (up to 70%) within a day, whereas the extent of insulin release from PLGA nanoparticles accounted for about 30−40% within 24 h (Yang et al., 2012).

BSA and immunoglobulin (IgG) bearing PEO−PLGA particles offered high encapsulation efficiency (EE = 58.9%) and slow *in vitro* release rate (Santander et al., 2010), which pointed out the potential viability of such commercial products.

High affinity of the core and wall materials is paramount to the formation of stable encapsulated peptide products that can withstand food processing and storage conditions with limited diffusion losses of the core materials. Contrary to EE, a recent study demonstrated that the release kinetics of peptides encapsulated in protein microbeads in aqueous environment was inversely proportional to the peptide hydrophobicity with average release rate constants of 0.1 and 0.014 min1 for Phe−Trp and Leu−Trp−Met−Arg−Phe, respectively, after 1 h. Conversely, the modification of rapeseed protein by acylation and high pressure treatment that resulted in higher EE was found to increase the % release of the encapsulated peptide compared with the native protein after 24 h using the dynamic dialysis method. This indicates weaker interaction of the peptides with the modified protein carriers. Although theoretically promising, the dearth of experimental information on the biostability of encapsulated protein hydrolysate and peptides makes it difficult to evaluate the prospects of encapsulation in oral delivery of bioactive peptides. A myriad of bioactive peptides derived from various food proteins have been reported, and it is becoming increasingly apparent that the focus needs to be shifted to the translation of the peptides into commercial functional food products.

Studies focused on characterizing the digestion and release of encapsulated peptides during gastrointestinal processing are crucial in understanding the effect of encapsulation on biostability. One study evaluated the biostability of bioactive peptides encapsulated with a carboxymethylated gum and sodium alginate, and found minimal (up to 10%) and maximal (up to 60%) release of protein materials after simulated gastric and intestinal digestion phases, respectively. The released peptides at the intestinal phase can then be presented for absorption into the enterocytes, and subsequently into

circulation where they are still susceptible to further peptidolytic modification. Therefore, it is imperative to assess the digestion kinetics and biostability of encapsulated peptides and their bioavailability in different physiological sites to ensure the release of intact bioactives at appropriate time and target location. Finally, the potential success of encapsulation applications depends on the fundamental characteristics of nanocarrier systems and the bioactive compound that they bear. There are rapid, robust, and reliable methods that can be used to characterize the basic characteristics of encapsulation systems such as particle size, morphology, wall thickness, chemical composition, and structure of the particle and the encapsulated material.

8.11 SAFETY OF NANOENCAPSULATION SYSTEMS LOADED WITH BIOLOGICAL MOLECULES

Nanotechnology has been gaining popularity in food industry in the recent years. However, consumers are suspicious about food products and packaging materials that contain nanocomponents and expect that either nanosystems should be excluded from food systems totally or not affect the human body in any negative way possible (Dowling, 2004).

Although there is a lack of long-term risks and regulatory inefficiencies, considerable variety of food products are in the market that contain nanomaterials, such as nutritional drinks, oils rich in vitamins and minerals on its micelles, etc. Toxicity issue is one of the major problems that must be investigated. There is strong possibility that certain nanocomponents can cause an increase of oxidative stress, generate free radicals, cancer, and DNA mutation (Moraru et al., 2003). Nanotechnological applications in food products require precautions related strongly to their impact on human health and environment (Chau, Wu, & Yen, 2007). Firstly, in order to minimize the possible side effects caused by nanosystem bearing food products, strong and reliable tests must be carried out that demonstrate conformity to food safety standards (Bowman & Hodge, 2007). In European (EU) regulations, food products containing nanomaterials are required to satisfy specific safety standards and testing procedures. In the USA, these products are regulated by the United States Food and Drug Administration (FDA). Also in Australia and New Zealand, Food Standards Australia and New Zealand (FSANZ) is responsible of regulating nanotechnological applications (Afroz et al., 2012).

8.12 CONCLUSION AND FURTHER REMARKS

Recently, there has been a considerable interest in the development of nanoscale delivery systems for biological molecules in order to improve the bioavailability and stability of such compounds. Consequently, studies

on nanoencapsulation and delivery of bioactives by polymeric and natural carrier based systems have been reviewed in the present work, including the preparation methods for efficient delivery of bioactives. Critical aspects of food compatible nanoencapsulation systems should pertain the analysis on toxicity characteristics, removal of any residual solvents, and the investigations on the biological fate of bioactives during digestion, absorption, and excretion of these systems.

Many beneficial food bioactives, such as proteins, peptides, enzymes and phytosterols, are known to be limited in terms of aqueous solubility, and consequently their inclusion in food systems is generally difficult. Especially regarding their unstable characteristics and tendency of degradation, delivery systems that are utilized in their implementation is of critical importance. The challenge for the food industry is the development of appropriate methods and techniques that can be used to manufacture these systems. Their utilization in foods has significant potential in the generation of novel functional foods.

Nanoassemblies could potentially alter the lipid digestibility characteristics of food products, supply the producers with novel tools to prepare functional foods with desirable sensory attributes, enhance the pH, heat, saline, oxidative, etc. stability of food components, tailor and control the release kinetics of bioactives from food matrices among other potential benefits. Although in most value added applications, some extra costs might be necessary to generate novel foods with enhanced features, industrial utilization seems to be steadily increasing which enhances the chances of novel applications in the marketplace. However, as of now, although there is an extended history of safe usage for natural encapsulation systems such as casein micelles, we cannot conclusively claim that the potential risks of nanomaterials to the health of humans are completely well-known and characterized.

Several technological and bioactive properties of peptides are characterized with an emphasis on functional food production. Especially their manufacture from protein-rich waste or by-products of industrial processing is currently being investigated in detail. Utilization of by-products can alter the economic difficulties in manufacture of bioactive added food products. In these areas, considerable extent of studies is still needed in order to figure out the specifics of cost-effective solutions that can generate a myriad of novel products and consequently, promote public health globally.

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