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Vegetable proteins in microencapsulation: a review of recent interventions and their effectiveness

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Abstract

Proteins from vegetable seeds are interesting for research at present because they are an abundant alternative to animal-based sources of proteins and petroleum-derived polymers. They are a renewable and biodegradable raw material with interesting functional and/or physico-chemical properties. In microencapsulation, these biopolymers are used as a wall forming material for a variety of active compounds. In most cases, two techniques of microencapsulation, spray-drying and coacervation, are used for the preparation of microparticles from vegetable proteins. Proteins extracted from soy bean, pea and wheat have already been studied as carrier materials for microparticles. These proteins could be suitable shell or matrix materials and show good process efficiency. Some other plant proteins, such as rice, oat or sunflower, with interesting functional properties could be investigated as potential matrices for microencapsulation.

Contents

1. Introduction

Microencapsulation consists of the isolation of active substances (in the liquid, solid or gas state), to obtain products with spherical form and micrometric size, in which the active material or core, is shielded by a membrane from the surrounding environment. This technique can be applied for different purposes: protecting sensitive substances from the surroundings, development of controlled release properties, masking of unpleasant taste and odor of the substances, dilution of core material when it must be used in very small amounts or transformation of liquid compounds into mobile solids. Microencapsulation allows the creation of a physical barrier between the core and wall materials and the protection of sensitive ingredients (flavors, antioxidants, polyunsaturated oils, vitamins, drugs…) from the external medium, particularly, moisture, pH and oxidation. The release of microparticle content at controlled rates can be triggered by shearing, solubilization, heating, pH or enzyme action. This technology has different applications in the food, biomedical, pharmaceutical and cosmetic industries as well as in agriculture and catalysis (Dubey et al., 2009). The structure of microparticles is generally classified into microcapsules with a single core surrounded by a

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layer of wall material; microspheres with the core dispersed in a continuous matrix network and more complex structures such as multilayer microcapsules or multishell microspheres (Figure 1). Various processes may be used to produce encapsulated ingredients (Augustin et al., 2006; Benita, 2006; Dubey et al., 2009; Gouin, 2004; Munin and Edwards-Lévy, 2011; Jyothi et al., 2010): spray-drying, spray-cooling/chilling, fluidized bed, coacervation/phase separation, gelation, solvent evaporation, supercritical fluid expansion, interfacial polymerization (polycondensation), emulsion polymerization and extrusion. Choice of microencapsulation technique for a particular process will depend on the size, biocompatibility and biodegradability of microparticles needed, the physico-chemical properties of core and coating, the microparticles' application, the proposed mechanism for active core release, and on the process costs.

(a)   **(b)**   **(c)**   **(d)**
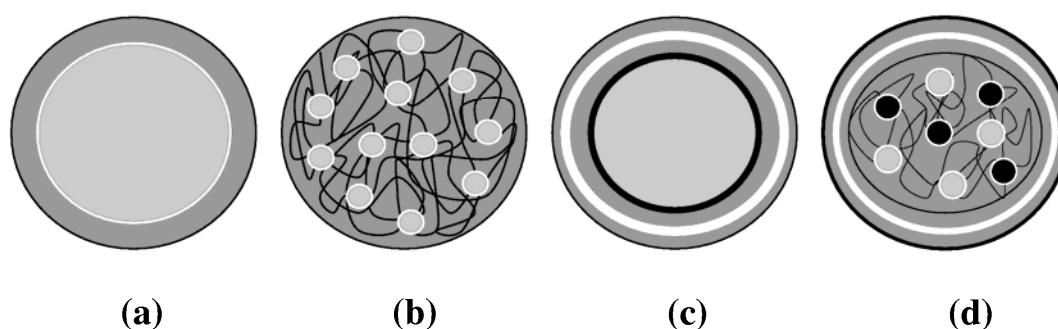
Fig. 1. Different morphologies of microparticles obtained by microencapsulation: (a) microcapsule, (b) microsphere, (c) multilayer microcapsule and (d) multishell and multicore microsphere.

Wall material particularly affects the microparticles' stability, the process efficiency and the degree of protection of the active core. Materials commonly used as carriers in the makeup of encapsulated ingredients, are synthetic polymers and co-polymers, and bio based materials such as carbohydrates, fats, waxes, and animal and plant derived proteins.

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Petroleum derived polymers commonly used in pharmacy and medicine as a matrix for microparticle preparation are polystyrenes, polyamides, polyurethanes, polyacrylates, phenolic polymers, and poly(ethylene glycols) (Dubey et al., 2009). Functionalization of polymeric chains makes it possible to obtain microparticles with new properties, different from those obtained with other wall materials, for example resistance to the action of chemical agents (Patel et al., 2010). Polysaccharides studied as a matrix for microencapsulation are starches (Jeon et al., 2003; Murúa-Pagola et al., 2009), maltodextrin (Krishnan et al., 2005; Saénz et al., 2009; Semyonov et al., 2010), gum arabic (Kim et al., 1996; Shaikh et al., 2006), pectin (Drusch, 2007; Gharsallaoui et al., 2010), chitosan (Higuera-Ciapara et al., 2004; Pedro et al., 2009), and alginates (Yoo et al., 2006; Huang et al., 2010; Wikstrom et al., 2008). The major advantages of these biopolymers are their good solubility in water and low viscosity at high concentrations, compared to proteins. Often carbohydrates are mixed with proteins (Augustin et al., 2006; Ducel et al., 2004b; Mendanha et al., 2009; Pereira et al., 2009; Pierucci et al., 2006; Pierucci et al., 2007; Yu et al., 2007) to improve the emulsifying and filmogenic properties during microencapsulation. Furthermore, protein-carbohydrate conjugates covalently cross-linked by the Maillard reaction had shown interesting functional properties (Augustin et al., 2006; Rusli et al., 2006). Various lipophilic substances such as glycerides, oils, phospholipids, carotenoids and waxes are also used as carrier materials in microencapsulation (Eldem et al., 1991; Lee et al., 2003; McClements et al., 2007; Muller et al., 2002; Patel et al., 2010). They permit barrier creation for the protection of sensitive ingredients against moisture, plus their transport in aqueous media.

Proteins extracted from animal derived products (whey proteins, gelatin, casein) and from vegetables (soy proteins, pea proteins, cereal proteins) are widely used for encapsulation of active substances. These natural polymers present several advantages: biocompatibility, biodegradability, good amphiphilic and functional properties such as water solubility, and

emulsifying and foaming capacity. The use of vegetable proteins as wall-forming materials in microencapsulation, reflects the present "green" trend in the pharmaceutical, cosmetics and food industries. In food applications, plant proteins are known to be less allergenic compared to animal derived proteins (Jenkins et al., 2007; Li et al., 2012). For these reasons, over the past few years, the development of new applications for plant products rich in proteins has became an increasingly interesting area for research. For the last decade, the protein ingredient industry has been turning towards plants as a preferred alternative to animal-based sources, e.g. in vegetarian diets, due to increased consumer concerns over the safety of animal-derived products (Jiménez-Yan et al., 2006; Sawashita et al., 2006; Choi et al., 2010). Currently, the widespread presence of microparticles based on animal proteins, contrasts with the very limited use of plant proteins in industry. This tendency should be reversed in coming years.

Vegetable proteins consist of several fractions: the major fraction is glutenin, soluble in alkaline water solutions; the globulin fraction, soluble in salt solutions, followed by the albumin and prolamin, fractions soluble in water and ethanol respectively (Osborne, 1909). Among vegetable proteins used as a wall material in microencapsulation, we find mainly soy protein isolate, pea protein isolate and cereal proteins. Soybean proteins have functional properties suitable for microencapsulation, such as solubility, water and fat absorption, emulsion stabilization, gelation, foaming, plus good film-forming and organoleptic properties (Franzen and Kinsella, 1976). Soy glycinin and conglycinin are somewhat similar (comparable molecular weights, amino acid composition, subunit structures) to pea legumin and vicilin (Koyoro and Powers, 1987). The globulins of pea protein have all the functional properties necessary for successful incorporation into microencapsulation systems as a wall material. The proteins of cereals (oat, wheat, barley and corn) are more advantageous from the nutritional standpoint, and they have attracted research and commercial attention for this

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reason. Due to their interesting functional properties and potential food applications, these proteins were also studied as wall material for microencapsulation (Ducel et al., 2005; Ducel et al., 2004b; Wang et al., 2011b). Sunflower proteins have particularly interesting thermal behavior, gelling properties and surface activity. Compared with other sources of vegetable proteins, sunflower seeds have been reported to have a low content in anti-nutritional factors. These proteins have often been compared to commercial soy proteins, extensively researched on functionality, with regard to their functional properties (Gonzalez-Perez and Vereijken, 2007).

This review presents the recent works dealing with the use of vegetable proteins in microencapsulation. The influence of the proteins functional properties as well as the microencapsulation technique on process efficiency and properties of obtained microparticles is particularly discussed.

2. The microencapsulation techniques applied to vegetable proteins

The two techniques mainly used for microencapsulation of active material by vegetable proteins are spray-drying and coacervation. Both processes share the aspect of "green chemistry" with vegetable proteins as renewable and biodegradable resources, plus, the two techniques do not need the use of organic solvents. Other processes such as gelation or solvent evaporation techniques can be also considered (Dubey et al., 2009; Gouin, 2004).

Spray-drying is a continuous process to convert an initial liquid into a solid powder of microparticles (Figure 2a). It is a very common dehydration process used to form a continuous matrix surrounding the active substances. The initial liquid (solution, emulsion or suspension) containing wall and core materials is sprayed into a stream of heated air. The solvent, almost always water, is evaporated to give instantaneous powder production. This technology offers several advantages: it is simple, relatively inexpensive, rapid and thus

widely used in industry. The important factor for successful microencapsulation by spray-drying is a high solubility of shell material in water (or other chosen solvent) and a low viscosity at high solid content. Disadvantages of this technique are loss of a significant amount of product (due to adhesion of the microparticles to the wall of the spray-dryer) and the possibility of degradation of sensitive products at high drying temperatures.
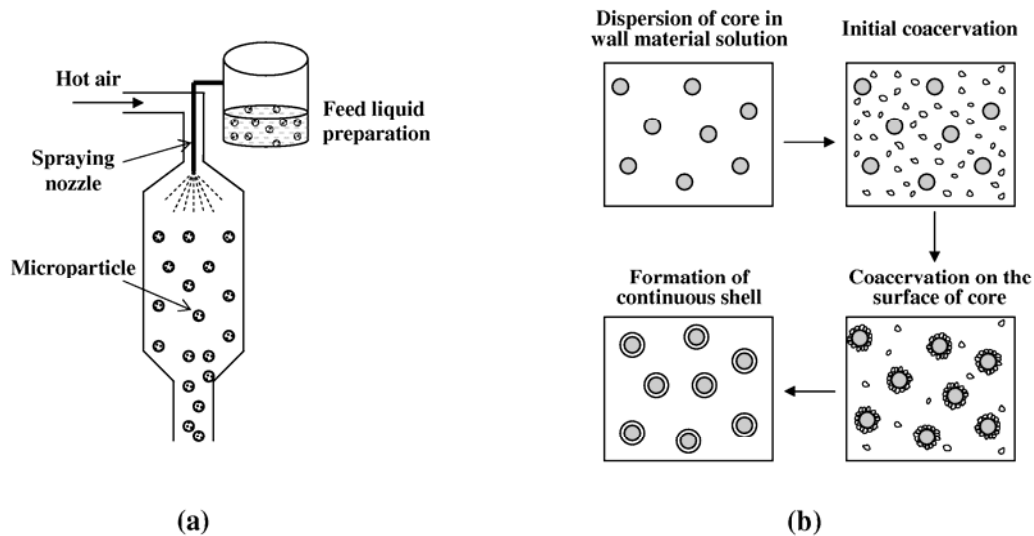
Fig. 2. Schematic representation of microencapsulation process by spray-drying (a) and coacervation (b) techniques. (Redrawn from Jyothi et al., 2010).

Microencapsulation by coacervation is carried out by precipitation of wall forming materials around the active core under the effect of one of the following factors: change of pH or temperature, addition of a non-solvent or electrolyte compound (Figure 2b). This controlled desolvation results in the formation of a polymeric network around the core. This shell of coacervates can be solidified using a chemical or enzymatic cross-linker (Gouin, 2004). Coacervation occurs either via a simple or complex method. Simple coacervation involves only one colloidal solute and thus formation of a single polymer envelope. Complex coacervation is produced by mixing two oppositely charged polyelectrolytes for shell formation around an active core (Wilson and Shah, 2007). Finally, one of the factors that

limits the use of coacervates in encapsulation is their sensitivity to pH and ionic strength (Augustin et al., 2006).

The important point to consider for the use of proteins in encapsulation systems is their instability in acid media. The isoelectric point of proteins means they are insoluble in most acidic systems and sensitive to precipitation with pH values lower than 7, especially when acidic core materials are used (e.g. ascorbic acid). For the majority of vegetable proteins in aqueous solution, the isoelectric point is located in a pH range between 3 and 5. For this reason these biopolymers are usually used in alkaline conditions in order to obtain good solubility of proteins and to efficiently encapsulate active substances.

Particle properties, such as morphology, size and releasing characteristics, can be very different depending on the process chosen. Two forms are mainly obtained by the spray-drying and coacervation method: microcapsules and microspheres (Figure 1). Particle size obtained by the spray-drying process is typically between 1 μm and 50 μm (Richard and Benoit, 2000) while size of particles obtained by the coacervation method can vary from nanometers to several hundred microns (Merodio et al., 2001; Bayomi et al., 1998; Gan et al., 2008).

These two processes give high values (up to 100%) of microencapsulation efficiency (MEE). The latter is defined as the ratio between the percentage of active core encapsulated in the powder and the percentage of active core in the initial liquid.

3. Vegetable proteins in microencapsulation

3.1. Soy proteins

Soy bean seeds contain an important fraction (35-40%) of proteins mainly glycinin and conglycin (50-90% of total proteins) (Ruiz-Henestrosa et al., 2007). The glycinin fraction (11S globulin) has a molecular weight of about 350 kDa while conglycin (7S globulin

fraction) is about 70 kDa. Isolated and purified soy proteins show interesting physico-chemical and functional attributes in particular gel-forming, emulsifying and surfactant properties (Gu et al., 2009). These protein characteristics and their solubility are strongly dependent on pH, heat treatment, and the presence and concentration of salts or other ingredients (oil, carbohydrate, surfactant).

Soy protein isolate (SPI) use in microencapsulation has already been studied by various authors (Table 1). SPI is generally used as an individual coating material, but can also be mixed with polysaccharides (Augustin et al., 2006; Rusli et al., 2006; Yu et al., 2007). The combination of proteins with carbohydrates as a carrier material favors better protection, oxidative stability and drying properties (Augustin et al., 2006). Due to SPI hydrosolubility, microparticles are mainly produced using the spray-drying technique (Augustin et al., 2006; Charve and Reineccius, 2009; Favaro-Trindade et al., 2010; Kim et al., 1996; Ortiz et al., 2009; Rascon et al., 2010; Rusli et al., 2006; Yu et al., 2007), but coacervation and gelation have also been investigated (Chen and Subirade, 2009; Gan et al., 2008; Lazko et al., 2004a; Lazko et al., 2004b; Mendanha et al., 2009; Nori et al., 2010).

Table 1. Microencapsulation with SPI-based wall material.

Microencapsulation process	Wall material	Core material	Reference
Spray-drying	SPI	Orange oil	Kim et al. (1996)
Spray-drying	Mixture of proteins and polysaccharides	Fish oil	Augustin et al. (2006)
Spray-drying	SPI/glucose syrup	Stearin, palme oil	Rusli et al. (2006)
Spray-drying	SPI/maltodextrin	Phospholipide	Yu et al. (2007)
Spray-drying	SPI	Flavors	Chavre and Reineccius (2009)
Spray-drying	SPI	Casein hydrolysate	Ortiz et al. (2009)
Spray-drying	SPI	Paprika oleoresin	Rascon et al. (2010)
Spray-drying	SPI/gelatin	Casein hydrolysate	Favaro-Trindane et al. (2010)
Spray-drying	SPI	α-tocopherol	Nesterenko et al. (2012)
Simple coacervation	SPI	Fish oil	Gan et al. (2008)
Simple coacervation	Soy glycinin	Hexadecane	Lazko et al. (2004a)
Complex coacervation	Soy glycinin/sodium dodecylsulfate	Hexadecane	Lazko et al. (2004b)
Complex coacervation	SPI/pectin	Casein hydrolysate	Mendanha et al. (2009)
Complex coacervation	SPI/pectin	Propolis	Nori et al. (2010)
Complex coacervation	SPI/gum arabic	Orange oil	Jun-xia et al. (2011)
Gelation	SPI	Riboflavin	Chen and Subirade (2009)

3.1.1. Microencapsulation by spray-drying

In the case of hydrophobic core microencapsulation, an oil-in-water emulsion is prepared before the encapsulation step (Augustin et al., 2006; Kim et al., 1996; Rascon et al., 2010; Rusli et al., 2006; Yu et al., 2007; Nesterenko et al., 2012). These emulsions are often carried out by high pressure homogenization because of its interesting results in terms of emulsion properties and stability (Gharsallaoui et al., 2007). Moreover, increasing homogenization pressure gives a slight decrease in oil droplet size (Rusli et al., 2006) and emulsion viscosity (Yu et al., 2007). Rusli et al. (2006) also noted a slight improvement in microencapsulation efficiency with an increase of homogenization pressure. Some results are given in Table 2.

Table 2. High pressure homogenization influence on the SPI-based emulsions properties.

Wall/core w/w	Pressure (MPa)	Droplet size (µm)	Viscosity (cP)	Reference
2/1	35	1.52	-	Kim et al. (1996)
2/1	35	0.57±0.1	-	Augustin et al. (2006)
1/1	18 → 35	0.45→0.32	-	Rusli et al. (2006)
1/1	15 → 55	-	100 → 70	Yu et al. (2007)
4/1	30	1.05±0.66	-	Rascon et al. (2010)
2/1	50	1.1±0.02	15	Nesterenko et al. (2012)

The intense mechanical forces undergone by globular proteins and oil droplets during homogenization, promotes oil droplet dispersion and protein structure modification (Rampon et al., 2003), such as unfolding of proteinic chains. This unfolding causes the exposure of polar and non polar protein regions, and movement of charged amino acids to new local environments and makes them more surface-active. The main changes are in the secondary

and tertiary structure that can modify the surface appearance of the amino acids. As proteins are the most surface-active components in the emulsion they accumulate at the oil-water interface, enwrapping the newly formed oil droplets. This can be explained by the tendency of proteins to adsorb more extensively and less reversibly at hydrophobic surfaces than at hydrophilic surfaces (Dickinson, 1999). The resulting stabilizing layer provides immediate protection of the fine droplets against re-coalescence and thus gives physical stability to the emulsion (Dickinson, 2001).

The solid level in the emulsion is also a key parameter influencing active core retention (Charve and Reineccius, 2009), and it has been shown that the MEE is improved with increasing solid content. This could be explained by the reduction of core molecule mobility in wall material and of the time needed to form the protective shell, both induced by a high solid content. On the other hand, past a critical concentration (generally 20% w/w solid content) an abrupt increase in viscosity is observed, which involves a significant fall in process efficiency (Yu et al., 2007).

From the process point of view, it has been shown that drying inlet temperature also affects the MEE. Indeed, a high drying temperature supports the formation of a rigid wall material shell on the microparticle surface, limiting core molecule migration and release (Rascon et al., 2010).

In the spray-drying microencapsulation process, efficiency can also be influenced by volatile properties of active core material, e.g. a decrease of MEE for encapsulation of volatile composites such as aromas compared to classic oils. Indeed, during the drying process, the liquid preparations are subjected to high temperatures (150-180°C) and this provokes core material evaporation, which explains the low efficiency of microencapsulation of citral (35%) (Charve and Reineccius, 2009) compared to stearin (91%) (Rusli et al., 2006) by SPI.

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In our recent study (Nesterenko et al., 2012), it has been shown that grafting of hydrophobic fatty acid chain to soy proteins by acylation can enhance the retention of hydrophobic active material (α-tocopherol) during microencapsulation by spray-drying. Process efficiency was improved from 79.7% to 94.8% when soy proteins were acylated with dodecanoyl chloride. Moreover, this increased retention efficiency after protein acylation was observed for different core/wall ratios, demonstrating that soy proteins in native and modified state represent a relevant encapsulant agent for hydrophobic substances.

3.1.2. Microencapsulation by coacervation

Soy proteins have also been studied in microencapsulation as wall materials using the coacervation method, and several parameters influencing the coacervation MEE have been found. Among them: the active core and wall material concentrations, the temperature and the pH of the media. When active hydrophobic core concentration exceeds 50% w/w, a decrease in process efficiency is generally observed (Lazko et al., 2004a; Mendanha et al., 2009; Rusli et al., 2006; Jun-xia et al., 2011). This phenomenon is particularly well illustrated in the work of Mendanha et al. (2009) where a change of wall/core ratio from 1/1 to 1/3 involved a decrease of MEE from 92% to 79%. Jun-xia et al. (2011) attributed this tendency to incomplete emulsification after addition of excessive oil in system. Unemulsified oil affected the electrostatic interactions between soy proteins and gum arabic, and thus emulsion destabilization.

The protein concentration (as wall material) during the emulsification step is strongly related to the stability and size of coacervates. This could be explained by the specific surface of oil droplets, which is inversely proportional to their mean diameter in emulsion (Lazko et al., 2004a). Due to protein surfactant properties, increasing protein concentration would result in an increase in oil droplet specific surface, improving the adsorption of proteins on the oil-

water interface and the droplets' coalescence resistance, and thus a decrease in their mean diameter (generally detected by light scattering). On the other hand, Lazko et al. (2004a) also demonstrated that protein concentration does not seem to have a significant influence on the microcapsule wall thickness.

Some authors (Lazko et al., 2004a) have noticed that microencapsulation by the coacervation method is more effective under acidic pH and high temperature conditions (pH 2 and 55°C respectively). Acid mediums favor 11S globular protein denaturation, characterized by deformation of their quaternary structure to secondary and tertiary structures. The overall accessibility of hydrophobic protein sites inside the spherical formations can be improved by this structure change (Magdassi, 1996; Wagner and Gueguen, 1995). At pH's below the isoelectric point, the protein COO^- functions become uncharged COOH groups, and protein hydrophilicity decreases. Thus, an acidic medium would favor the affinity between proteins and the hydrophobic active core in the emulsions, which should result in improved MEE.

When microencapsulation is undertaken by coacervation, a cross-linking step is often added at the end of the process, mainly to reinforce the microcapsule shells. This supplementary step would not affect the efficiency of protein precipitation around oil droplets, but would play an important role in emulsion stability, and consequently on microcapsule size and dispersion, particularly with prolonged stirring. Without reticulation, the coalescence of microcapsules was observed, resulting in a significant increase in microcapsule average diameter (from 90 μm to more than 200 μm), whereas this coalescence is absent when microcapsules were cross-linked (no change in microcapsule diameter was observed) (Lazko et al., 2004a). Glutaraldehyde is the most commonly used cross-linking agent, allowing stable microcapsule dispersion to be obtained over time (Lazko et al., 2004b), plus better mechanical properties. But glutaraldehyde is a relatively toxic product, which limits its use in applications such as the food industry (Leung, 2001).

In an effort to mask the bitter taste of some hydrophobic hydrolysates (e.g. casein hydrolysate) and be able to incorporate them into food products, their microencapsulation by soy proteins was investigated by spray-drying (Favaro-Trindade et al., 2010; Ortiz et al., 2009) and coacervation (Mendanha et al., 2009) techniques. During encapsulation, the hydrophobic interactions between casein hydrolysate and soy proteins lessen the undesirable taste of casein. In both cases, the authors demonstrate the decrease in microencapsulation efficiency and the increase in particle size with increasing active core concentration.

Chen and Subirade (2009) reported the preparation of soy protein based microspheres by cold gelation method (initiated by glacial acetic acid in the presence of calcium carbonate) to elaborate delivery systems for nutraceutical products (riboflavin). Obtained microparticles had spherical morphology with the diameter about 15 µm. Active material was efficiently encapsulated by soy proteins (process efficiency of 79-88%) at ambient temperature without using cross-linking reagents, which could be interesting for various food and pharmaceutical applications.

Overall, numerous studies have shown the abilities of SPI as an encapsulating agent, using both spray-drying and coacervation techniques. In both methods, specific parameters affect microencapsulation efficiency and microparticle size, particularly the active core concentration (Lazko et al., 2004a; Mendanha et al., 2009; Rusli et al., 2006; Yu et al., 2007), but authors showed that high values of MEE could be attained in both cases, by using suitable experimental conditions. The main differences between these two methods are the structure of the microparticles obtained, and consequently the release of the active core, and the microparticle sizes, usually higher with coacervation (less than 100 µm for spray dried microparticles instead of generally more than 100 µm for coacervated microcapsules).

3.2. Pea proteins

Pea proteins are extracted from pea seeds where they represent a 20% to 30% fraction including mainly globulins (65-80%) and two minority fractions, albumins and glutelins. Globulins comprise three different proteins – legumin, vicilin and convicilin (Koyoro and Powers, 1987). Pea legumin represents the 11S globulin fraction with a molar mass between 350 and 400 kDa, while vicilin and convicilin represent the 7S globulin fraction with a molar mass of about 150 kDa.

Pea proteins extracted from grains possess interesting gel-forming (Akintayo et al., 1999) and emulsifying (Raymundo et al., 2005) properties. However, in the literature for microencapsulation uses, these proteins are generally associated with polysaccharides (Ducel et al., 2004b; Gharsallaoui et al., 2010; Pereira et al., 2009; Pierucci et al., 2006; Pierucci et al., 2007). Indeed, polysaccharide/protein interactions give new functions to pea proteins without chemical or enzymatic modification, particularly solubility, foaming and surfactant properties (Liu et al., 2010). These interactions can also create stable emulsions and thus give better particle size distribution and improve the efficiency of the microencapsulation process. Table 3 shows various pea protein/polysaccharide pairs and the different processes used for active core microencapsulation.

Table 3. Microencapsulation based on the complexes pea protein/polysaccharide as a wall material.

Microencapsulation process	Wall material		Core material	Reference
	Protein	Polysaccharide		
Complex coacervation	Pea globulins	Gum arabic Carboxy-methylcellulose Sodium alginate	Triglyceride	Ducel et al. (2004)
Spray-drying	Pea proteins	Maltodextrin	Ascorbic acid	Pierrucci et al. (2006)
Spray-drying	Pea proteins	Maltodextrin	α-tocopherol	Pierrucci et al. (2007)
Spray-drying	Pea proteins	Maltodextrin	Ascorbic acid	Pereira et al. (2009)
Spray-drying	Pea proteins (as additive)	Pectin Maltodextrin	Triglyceride	Gharsallaoui et al. (2010)

3.2.1. Microencapsulation by spray-drying

Several studies deal with pea proteins as wall material for microencapsulation, using the spray-drying technique. The main properties of the microparticles obtained are summarized in Table 4. Utilization of protein/polysaccharide mixtures allows the possibility of combining the specific properties of each of these polymers. Polysaccharide products possess low emulsification properties compared to proteins, and are usually used as wall materials in the presence of a surface-active constituent. The incorporation of carbohydrates with proteins as the encapsulated matrix, gives increased emulsion stability and better protection of active ingredients against oxidation (Young et al., 1993). Gharsallaoui et al. (2010) noted that in protein/carbohydrate blends, proteins serve as an emulsifying and film-forming agent, while polysaccharides act as a matrix forming material. The retention of the

active core observed in pea protein microparticles is similar to (or better than) in the particles with a protein/carbohydrate mixture. This, presumably, is induced by electrostatic interactions between proteins and encapsulated material (Pierucci et al., 2007). As stated in the literature, the addition of maltodextrin to the pea protein wall material increases the particle size, in particular for a hydrosoluble active material (Pierucci et al., 2006). The authors justified this increase by the fact that maltodextrin can induce the rapid formation of a glassy surface which would allow air expansion inside microparticles, giving an increase in particle diameter.

Table 4. The properties of microparticles produced by spray-drying with the pea proteins as a wall material (wall/core ratio of 2/1 w/w).

Wall material	Core material	Microparticle size (μm)	MEE (%)	Core amount (g/100 g powder)	Reference
Pea proteins	α-tocopherol	2.2	86.8	28	Pierrucci et al. (2007)
Pea proteins/ maltodextrin (1/1, w/w)	α-tocopherol	3.5	77.8	25	
Pea proteins	Ascorbic acid	2.7	101.9	34.6	Pierrucci et al. (2006)
Pea proteins/ maltodextrin (1/1, w/w)	Ascorbic acid	8.2	95.9	29	

The results reported, demonstrate that pea proteins alone or in association with polysaccharides are totally appropriate for the microencapsulation of hydrophilic (ascorbic acid (Pereira et al., 2009; Pierucci et al., 2006)) hydrophobic (α-tocopherol (Pierucci et al., 2007), and triglyceride (Ducel et al., 2004b; Gharsallaoui et al., 2010)) active core materials.

3.2.2. Microencapsulation by coacervation

Ducel et al. (2004) examined the use of pea globulin (isoelectric point in a pH range 4.4-4.6) for triglyceride microencapsulation by complex coacervation (Table 3), plus the influence of pH and polymer concentration on the microcapsule size. Increasing pea globulin/ gum arabic (50:50) blend concentration in the initial makeup, resulted in increased microcapsule size. For example, at pH 3.5, microcapsule diameters varied from 28 µm to 97 µm with a concentration change of 1 g/L to 10 g/L respectively. Conversely, Lazko et al. (2004a) observed a decrease of coacervate size with an increase of soy protein concentration. In fact, the mean diameter of microparticles obtained, decreased from 153 µm to 88 µm as the protein concentration increased from 0.5 g/L to 5 g/L respectively. This discordance between published results was probably due to coacervation process differences. Complex coacervation was used in the case of pea proteins, and particle agglomeration and coalescence increased their size. The presence of polysaccharides in the initial preparation can also influence coacervate agglomeration (Klassen and Nickerson, 2012). On the other hand, simple coacervation was used for preparing soy protein microparticles. Higher concentrations of surface active protein in the emulsion increased the coalescence resistant coacervates. In addition, the two coacervation processes were not made under exactly the same conditions, for example temperatures of 30°C and 55°C, pH values of 3.5 and 2.0, mechanical stirring at 500 rpm and magnetic stirring at 600 rpm, for pea proteins and soy proteins respectively.

It is apparently also possible to prepare microparticles from one fraction of pea proteins (legumin (Irache et al., 1995) or vicilin (Ezpeleta et al., 1997; Ezpeleta et al., 1996a)) without polysaccharide addition, and to coat an active material, and these studies involved microparticle preparation (with legumin or vicilin) using simple coacervation. Concerning particle size distribution, the mean particle diameter varied from 200 to 700 nm. Finally, the average sizes of microcapsules based on pea proteins obtained by coacervation, varied from

about 10, to hundreds of microns, while for spray-dried microspheres, the average size is always less than or near 10 µm.

3.2.3. Pea proteins as an additive for microencapsulating systems

The emulsifying properties of pea proteins (Ducel et al., 2004a) make them potentially useful as an additive to improve emulsion stabilization instead of as a simple main wall material. Gharsallaoui et al. (2010) used a small amount of pea protein (0.5% w/w) as an emulsifier to form oil-in-water emulsion containing small oil droplets. Then pectin and maltodextrin were added, to produce an emulsion containing triglyceride droplets coated with protein-polysaccharide membranes. This study confirmed the interest of combining the properties of polysaccharides with those of proteins. The emulsions with polysaccharides seemed to be less sensitive to pH variations, high ionic strengths and high temperatures, than those with only proteins (Dickinson, 2003; McClements, 1999). In addition, the hydrophobic polypeptides of proteins, added to polysaccharide based emulsions, have a high capacity to adsorb at the oil-water interfaces, (for example pea globulin at acid pH) and thus to stabilize emulsions (Gharsallaoui et al., 2010). These studies show that the use of small quantities of protein could stabilize emulsions against coalescence, pH and temperature variations.

To sum up, pea extracted proteins show convenient encapsulating properties and are used for active material protection or for emulsion stabilization. Properties of the resulting microparticles were dependent on the microencapsulation technique used, process conditions and the use of additives such as polysaccharides.

3.3. Wheat proteins and other cereal proteins

Wheat contains a specific protein: gluten, obtained as a by-product during starch isolation from wheat flour. The latter is a complex material, composed of proteins and a small

polysaccharide fraction. Its two main components are gliadin and glutenin. Gliadin is composed of single chain polypeptides, with an average molecular weight of 25-100 kDa, linked by intramolecular disulfide bonds, and soluble in neutral 70% ethanol. Glutenin is a hydrosoluble fraction consisting of gliadin-like subunits stabilized by intermolecular disulfide bonds in large aggregates, with a molecular weight greater than 105 kDa (Bietz and Rothfus, 1970).

Gluten represents approximately 80% of wheat seed proteins, plays an important role in wheat flour quality (Day et al., 2006), and is used essentially as a human and animal food source. While its insoluble nature is an important property for traditional applications, particularly in bread and baked products, this insolubility in water limits its use in many other applications such as cosmetics and drugs.

Wheat gluten is the cereal protein most studied in the microencapsulation field (Ducel et al., 2005; Ducel et al., 2004b; Ezpeleta et al., 1996b; Iwami et al., 1987; Mauguet et al., 2002; Yu and Lee, 1997). Its low water solubility and its viscoelasticity provide this plant polymer with various interesting physico-chemical characteristics, such as gel- and film-forming properties (Sun et al., 2009). Wheat proteins alone, or in combination with polysaccharides are good for encapsulating active core materials using various techniques. Some studies have also been made with other cereal proteins as a wall material: barley protein or corn zein (Parris et al., 2005; Wang et al., 2011a; Wang et al., 2011b; Zhong et al., 2009; Patel et al., 2012). Barley proteins, studied by Wang et al. (2011b), are composed of two protein fractions: glutelin and hordein. Both these fractions show excellent film-forming and emulsifying properties (Wang et al., 2011a). Corn extracted prolamin – zein is a protein fraction soluble in hydro-alcoholic solutions and well-known for its good filmogenic properties (Beck et al., 1996). Table 5 summarizes microencapsulation studies with these biopolymers.

Table 5. Cereal proteins in the microencapsulation

Microencapsulation process	Wall material	Core material	Reference
Spray-drying	Wheat gliadin, corn zeine	Linoleic acid	Iwami et al. (1987)
Spray-drying	Barley protein	Fish oil	Wang et al. (2011a,b)
Simple coacervation	Gluten/casein	Pyrrolnitrin	Yu and Lee (1997)
Simple coacervation	Gliadin	Hexadecane	Mauguet et al. (2002)
Complex coacervation	α-Gliadin/arabic gum	Vaselin oil	Ducel et al. (2004, 2005)
Solvent evaporation	Gliadin	Retinoic acid	Ezpeleta et al. (1996)
Solvent evaporation	Gluten/poly(ethylen oxide)	Diltiazem hydrochloride	Andreani et al. (2009)
Phase separation	Corn zein	Essential oils	Parris et al. (2005)
Supercritical anti-solvent process	Corn zein	Lysozyme	Zhong et al. (2009)
Anti-solvent precipitation method	Corn zein	Quercetin	Patel et al. (2012)

3.3.1. Microencapsulation by coacervation

Ducel et al. (2005) studied the encapsulation of vaseline oil with a gliadin/ gum arabic wall, by complex coacervation, focusing on protein concentration and effect of pH on microcapsule properties. They found that a decrease in medium pH (from 3.5 to 3) gave an increase in viscoelasticity and a decrease in microcapsule average size (from 50 µm to 25 µm). The wall polymer distribution on the droplet surface was more homogeneous so particle aggregation was reduced. Encapsulation conditions and efficiency were improved by the pH decrease. Analogous behavior for a pea protein/ gum arabic encapsulating system, has been

observed (Ducel et al., 2004), and this result agrees with observations by Lazko et al. (2004a) concerning complex coacervation microencapsulation using soy glycinin. The range of wheat protein microcapsule size using coacervation (simple or complex) can vary from a few to two hundred micrometers (Ducel et al., 2005; Mauguet et al., 2002; Yu et al., 2007). These values are in line with those obtained from pea protein and soy protein microcapsules (Ducel et al., 2004b; Lazko et al., 2004a).

3.3.2. Microencapsulation using other processes

Only a few studies deal with wheat proteins for spray drying microencapsulation. Iwami et al. (1987) reported the encapsulation of linoleic acid in a gliadin matrix to improve its stability and digestibility, particularly for bread making applications. Wheat proteins were also used as wall material for microencapsulation with the solvent evaporation method (Andreani et al., 2009; Ezpeleta et al., 1996b), and proteins extracted from barley seeds were used as carrier material for fish oil microencapsulation by Wang et al. (2011). The spray-drying method was used for microparticle preparation with an inlet temperature of 150°C. The authors demonstrated 97-100% encapsulation efficiency and high oil content in the powder – around 50%. The barley protein microparticles obtained have a spherical shape and porous inner structure with diameters ranging from 1 to 5 µm. These proteins had a good capacity for protecting fish oil against oxidation in food preparations.

Andreani et al. (2009) worked on wheat gluten microspheres for the controlled release of a model drug (diltiazem), and evaluated the effect of a small amount of poly(ethylene oxide) on microsphere properties. They demonstrated that perfectly spherical porous microspheres could be obtained, with mean particle diameters of between 10 and 20µm, and encapsulation efficiency from 73% to 97%. They showed that the addition of 5% w/w of PEO to the gluten matrix improved the MEE significantly. This is probably due to higher porosity of

microparticles with PEO, and therefore greater specific surface area favoring better incorporation of the active core material. The effect of nature of solvent was studied by Ezpeleta et al. (1996b), They observed significant variations in microparticle diameter, according to solvent composition, highlighting the influence of physico-chemical interactions between proteins and solvents. Antimicrobial chicken egg white lysozyme, was encapsulated by zein protein using a supercritical anti-solvent process (Zhong et al., 2009), and heterogeneously sized microparticles, ranging from a few to 50 µm and 46.5% MEE were obtained. The active material release kinetics showed very promising microparticle properties for use in food production.

In short, cereal proteins are relevant biomaterials as a matrix for microencapsulation. They perform well for microencapsulation of hydrophobic and hydrophilic compounds alone, as well as mixed with polysaccharides or synthetic polymers.

3.4. Other vegetable proteins potentially useful in microencapsulation

Other proteins have properties making them possible contenders as wall material in microencapsulation, and this is especially true for rice proteins, oat proteins and sunflower proteins. Rice and oat proteins already have a large range of applications in the food sector. However, the physico-chemical properties of sunflower proteins have been extensively studied, and this natural polymer has no major industrial uses, meaning that it would be interesting to find new applications and develop high added-value products based on them.

3.4.1. Rice proteins

Rice is among the most important cereal crops in the world. It is established as the basic foodstuff for over half the world's population. Containing from 12 to 20% proteins, rice bran, mainly removed from the grain during the milling process to produce white rice, may be a

potential source of inexpensive high quality proteins (Hamada, 2000). Compared to rice bran, the protein content in rice grains is slightly lower, varying from 6 to 15% (Bienvenido, 1994). Rice proteins are generaly prepared by alkali extraction followed by isoelectric precipitation (Kaewka et al., 2009; Pinciroli et al., 2009) and by subcritical water treatement (Hata et al., 2008; Sereewatthanawut et al., 2008). In addition, rice has also been studied for the production of starch, monosodium glutamate, pigments and rice wine; thus rice protein could be an additional by-product to be exploited (Cao et al., 2009). After the sequential extraction of rice protein fractions, the following distribution has been obtained: about 75% glutenin, 15% globulin, 6% albumin and 3% prolamin (Agboola et al., 2005).

Chandi et al. (2007) analyzed the functional properties of rice protein concentrate (55% of the protein fraction). They noticed the excellent foaming stability lasting several days, the high emulsifying capacity in sugar based (5-15% w/w) solutions, and the good stability of emulsions depending on the pH and salt/sugar presence. The physico-chemical properties are similar to those of casein (Chandi and Sogi, 2007).

Rice bran isolate containing approximately 92% protein is prepared from defatted rice bran and its properties have been studied (Wang M et al., 1999). They showed that: the foaming properties of rice protein are similar to those of albumin from egg white; the emulsifying capacities of albumin from bovine serum (BSA) are significantly higher than those of rice proteins; minimum protein solubility is close to the isoelectric point at pH 4 and the maximum at pH 10; the main amino acid content of rice proteins is similar to that of casein and soy proteins; the denaturation temperature of rice protein isolate is about 83.4°C.

Rice proteins also associate well with polysaccharides (alginate and carrageenan) to form complex precipitates with possible new industrial applications (Fabian et al., 2010). From these results, the physico-chemical properties of rice proteins could provide favorable characteristics for wall material in microencapsulation. However overall, rice protein use

concerns the food industry, rather than potential low volume, high added value applications of microencapsulation.

3.4.2. Oat proteins

Oats is one of the most popular cereals for human and animal foods because of its high protein and fatty acid content. Protein content in oat grain is one of the highest, varying from 12 to 24% (Chronakis et al., 2004). The average amino acid composition of oat proteins is very attractive from a nutritional value point of view, and this is probably related to the higher proportion of albumins and globulins compared to proteins from the other cereal grains. Globulin represents the major part of oat proteins (around 70-80%). Oat protein concentrate has poor solubility and functional properties. To improve these physico-chemical properties, modifications such as enzymatic hydrolysis (Yao et al., 2007), acetylation and succinylation (Mohamed et al., 2009) were carried out, and demonstrated that these chemical modifications could improve the solubility, emulsifying activity and foaming capacity of oat proteins.

In conclusion, oat native proteins do not offer the required properties to be used in microencapsulation, but some specific modifications could allow them to be considered as wall materials.

3.4.3. Sunflower proteins

Sunflowers are mainly cultivated for the production of oil extracted from their seeds, and they are one of the major sources of edible oil. Proteins are the majority constituents in sunflower oil cakes, valued essentially as animal feed. The defatted sunflower flour contains a high quantity of proteins, around 27% in dry weight (Ordonez et al., 2001). The dehulled seed consists of about 20-40% crude protein, this value being highly affected by sunflower variety

(Gonzalez-Perez and Vereijken, 2007). The quantity of proteins extracted from the sunflower, also varies according to used solvent (mainly aqueous solutions) and the extraction conditions (stirring mode, temperature, pH). In the sunflower oil cake, four fractions of proteins are present (Linden, 1994): globulins constitute the main fraction ranging from 55 to 60%; albumins account for about 17-23% of total proteins and two minor fractions glutelins and prolamins give 11-17% and 1-4% protein fractions respectively.

In terms of sedimentation coefficients, sunflower proteins show two major fractions: the 11S globulins (also named helianthinin) and the 2S albumins. Helianthinin has been reported to be present as a globular oligomeric protein with a molecular weight of 300-350 kDa (Gonzalez-Perez and Vereijken, 2007), and this protein mainly exists in the 11S form (hexametric structure). Depending on pH, ionic strength, temperature and protein concentration, helianthinin may also occur in the 15-18S, 7S or 3S forms. In 11S sunflower proteins, different subunits are traditionally processed to give an acidic and a basic polypeptide linked by a single disulfide bond. These basic and acidic polypeptides range in molecular weight from about 21 to 27 kDa and from about 32 to 44 kDa respectively. The solubility of helianthinin with a minimum of 4-5.5 depends strongly on pH and ionic strength. Albumin proteins from sunflower, with a sedimentation coefficient of approximately 2S and molecular weights ranging from 10 to 18 kDa, show good solubility in aqueous solutions, independent of pH and ionic strength. Contrary to the majority fractions, the functional properties of glutelins and prolamins from sunflower seeds have not been reported in the literature.

The amino acid composition of soy proteins (Kovalenko et al., 2006) and sunflower proteins (Conde et al., 2005) are shown in Figure 3. Some similarities in total amino acid content for these vegetable proteins can be seen. The physico-chemical properties of sunflower proteins have already been studied (Gonzalez-Perez et al., 2005; Molina et al.,

2004; Patino et al., 2007). Most authors showed that sunflower preparations have better (or at least similar) emulsifying properties as those of soy protein preparations. The main results of these studies showed that the highest emulsifying capacity is observed in the pH range of 7-8 and the minimum at the isoelectric pH of 4.3; the extraction method and solvent used for protein extraction does not change the emulsifying ability of proteins; heating involving protein denaturation, increases the stability of emulsions but reduces their emulsifying capacities. This latter observation can be explained by the change of protein structure during heating denaturation, favoring chain unfolding and increased conformational flexibility. Thus the surface-active capacity of unfolded sunflower proteins becomes lower during emulsion formation, but after emulsion preparation it stays stable longer.
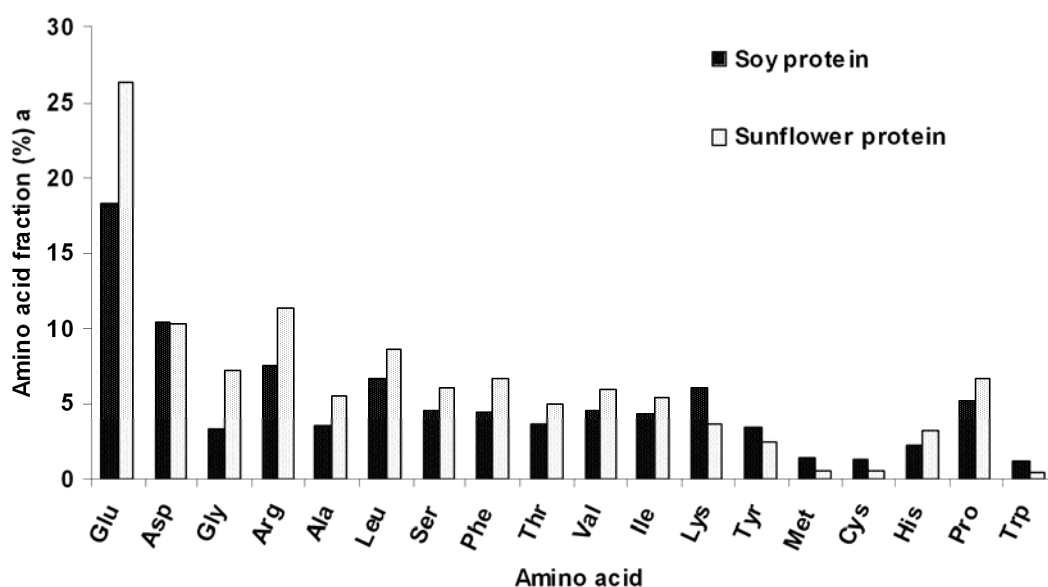

Fig. 3. Amino acid composition of soy (Kovalenko et al., 2006) and sunflower (Conde et al., 2005) proteins, every amino acid fraction is presented in g/100g of protein isolate.

Concerning foam properties, sunflower proteins seemed to be less efficient at forming foam than soy proteins. Nevertheless, sunflower protein foams are stable over time at a basic pH and a high concentration. Chemical modifications (for example enzymatic hydrolysis) of

sunflower proteins could lead to an improvement in their functional properties and to new interesting applications (Conde and Patino, 2007). The presence of phenolic compounds in sunflower proteins, which cause the green-brown color of its powder, limits their development as a source of food proteins for humans. Therefore, there could be very interesting new openings for these proteins in non-food industrial sectors. Microencapsulation could be one possibility for an industrial application of this agricultural by-product.

4. Industrial applications of microencapsulation by vegetable proteins

Pea proteins show a good properties for their potential application, in particular for the production of adhesives, bioplastics, emulsifiers and wall forming materials for microencapsulation (De Graaf et al., 2001). However, these proteins are no suggested to be used in technical applications. The functional properties of wheat proteins and corn zein also suggest several potential applications for these natural polymers in the fields of adhesives, matrix materials for microencapsulation, textiles, cosmetics and biodegradable plastics (Shukla and Cheryan, 2001). For both of these proteins, there is still no actual industrial application in microencapsulation, but they are potentially good candidates.

Conversely, soy bean proteins are already used as wall forming materials in the food industry, in particular to mask the undesirable taste of some nutritional additives (bioactive compounds for athletes, such as casein hydrolysate) (Favaro-Trindade et al., 2010; Mendanha et al., 2009; Ortiz et al., 2009; Sun-Waterhouse and Wadhwa, 2012) or to protect components sensitive to oxidation and\or volatile aromas (orange oil) (Gharsallaoui et al., 2007; Kim et al., 1996; Xiao et al., 2011).

5. Conclusions and future prospects

The use of vegetable proteins as a wall material for microencapsulation of various sensitive materials, reflects the actual "green" tendency in the food, pharmaceutical and cosmetics industries. The two main techniques used for microencapsulation of different core substances by these natural polymers, are spray-drying and coacervation. Particle morphology is very dependent on the process chosen, mainly because coacervation produces microcapsules, whereas microspheres are generally obtained with spray-drying. Vegetable proteins widely used as encapsulants are pea protein isolate, soy protein isolate, wheat gliadins, corn zein and barley protein. The various studies have proved the ability of proteins to efficiently protect different forms of active materials (hydrophilic or hydrophobic, solid or liquid) as an encapsulating agent, using both spray-drying and coacervation methods. However, microencapsulation efficiency, preparation stability and microparticle size could be affected by different parameters, such as active core and wall material concentrations, temperature and pH of media, encapsulation technique, use of additives or proteins combined with polysaccharides.

Other inexpensive proteins extracted from rice, oat or sunflower seeds are known for their interesting functional properties and could be suitable microencapsulation wall forming materials. These natural polymers show good solubility, emulsion forming ability and foaming stability, giving them the appropriate characteristics for potential use as efficient coating materials. Moreover, they can be associated with polysaccharides as is commonly the case in microencapsulation. Thus, the good physico-chemical properties of all these vegetable proteins open a new path for specific applications, the development of innovative delivery systems, and/or functional food products.

Some limitations of vegetable protein use for making high added value products could be the extraction cost to obtain high-quality proteins, low solubility of some proteins and

large polydispersity in the size of naturally occurring protein chains. Compared to other bio based materials for microencapsulation, such as polysaccharides, synthetic polymers or animal-based proteins, plant extracted vegetable proteins represent a very promising source of polymers with interesting functional properties. Their use as a wall material augurs well for the encapsulation of hydrophilic and hydrophobic substances by different techniques, and production of microparticles, with good microencapsulation efficiency and various potential applications.

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