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REVIEW

Vegetable proteins as potential encapsulation agents: a review

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Abstract

Proteins from plant sources are macromolecules of industrial interest due to its high availability, biodegradability, renewable character and functional properties such as biocompatibility, good amphiphilic properties, water solubility, foaming, emulsifying, gelling and film-forming abilities. In this scenario, these macromolecules have a high applicability in various emulsification stabilization processes, and have a potential use as a wall-forming material for the encapsulation processes of active ingredients. Currently, proteins extracted from soybean, pea, sunflower, rice and wheat seeds have already been studied as versatile stabilizers and emulsion coatings by coacervation and encapsulation processes by spray-drying. However, the capsule-forming ability of these macromolecules is enhanced by physical, chemical or enzymatic changes in their structure, favoring the interactions with the core material, the solvent, and increase of the encapsulation efficiency and its versatility in other encapsulation techniques.

1. Introduction

Encapsulation is defined as a mechanical, chemical or physicochemical process that isolates and protects the active ingredients potentially sensitive active ingredients to conditions such as oxygen, light, pH and other characteristics that affect the activity of the compound. This active ingredient can be liquid, solid or gaseous. (Quintero *et al.*, 2017). In this process, the polymer that protects the active is referred as the coating material, which controls the release and stability of the active ingredient (Jain *et al.*, 2015). In the encapsulation process, unlike the immobilization process, the coating material covers completely the active ingredient, whereas in the immobilization process some degree of exposure to the environment might occur (Kent and Doherty, 2014).

Microencapsulation is used for different reasons such as the stabilization of sensitive ingredients, protecting these compounds from the deleterious effects of the surrounding environment such as oxidation, extreme temperature, humidity or pH changes. This technique reduces the evaporation rate of volatile active ingredients from the core to the outside. Further, it modifies the release characteristics of the labile compounds, favoring its dilution properties, in some cases, generating a modulated release. In addition, the technique to mask the undesirable flavors and odors of the active ingredient (Lia *et al.,* 2015; Zhang, Li, Liu *et al.,* 2015).

In recent years, the challenges of drug controlled release, additive effectiveness, and optimum dosing have made the encapsulation process a fascinating alternative in several fields (Cardona and Cabrera, 2010). Therefore, the scientific output related to the encapsulation process has shown a remarkable increase in the previous decade, and several authors have been focused on the search for novel delivery systems, including the development of new coating materials.

The search for potential encapsulation agents has led to the development of plentiful polymers and copolymers, according to their different physico-chemical properties. As a result, new intelligent, versatile and selective systems (i.e., pH, or temperature driven systems) have been created to modulate the release of bioactive agents maximizing their functionality (Wang *et al.*, 2014). Nonetheless, most of these synthetic materials are not recognized as safe (GRAS) by the Food and Drug Administration (FDA). As a result, their use in the food, pharmaceutical, and cosmetic fields is limited (Avramenko *et al.,* 2016).

Currently, researchers have turned their attention towards polymers of natural origin since most of them

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are considered as GRAS. Thus, polysaccharides such as starch, maltodextrin (Semyonov *et al*., 2010), gums (Gharsallaoui *et al*., 2012), pectin (Gharsallaoui *et al*., 2012), chitosan (Gharsallaoui *et al*., 2012), among others (Avramenko *et al*., 2016) have been used as encapsulation agents. Proteins are versatile macromolecules due to their ability to self-associate, their dynamic structure, biocompatibility, amphiphilic properties and biodegradability; awarding it technical-functional properties such as emulsifying, foaming and gelling agents (Li and Tang, 2013; Zhang *et al.,* 2014; Wan *et al*., 2015; Avramenko *et al.,* 2016; Chang *et al*., 2016). Their chemical and structural versatility makes them suitable candidates for the delivery of bioactive ingredients (Wan *et al.,* 2015). However, only few proteins have been used as effective coating materials for the encapsulation of active ingredients, and to a lesser extent proteins from plant sources (Li and Tang, 2013). In recent years, the study of the encapsulation capability of plant proteins has increased since they have excellent physicochemical properties, and are considered as biodegradable, renewable, highly available, good coating materials, their production entails less consumption of natural resources, and they are considered as "environmentally economic" (Li and Tang, 2013). In this scenario, proteins obtained from soy, beans, peas, chickpeas, oats, corn, rice and sunflower seeds are included among the most studied proteins from plant sources. In fact, native proteins or their derivatives are considered as good coating materials, and as good alternative to proteins derived from animal sources and synthetic polymers for the encapsulation of several compounds (Pierucci *et al*., 2006; Li and Tang, 2013; Liu *et al.,* 2014; O'Neill *et al*., 2015; Wan *et al.,* 2015; Piornos *et al*., 2017). Therefore, this review is focused on recent available literature related to proteins as coating materials in various encapsulation processes, emphasizing the usefulness of vegetable proteins in this process. Likewise, a peer discussion of the encapsulation principles, techniques, physicochemical treatments and encapsulating capacity of vegetable proteins is also addressed. In order to develop this chapter, the literature available in the ScienceDirect, SpringerLink, Google Scholar, SciFinder and Scopus databases were reviewed between 2006 and 2016. Encapsulation or microencapsulation and vegetable proteins were used as keywords. Subsequently, each paper was completely revised, and only those papers containing the relevant information were included in this review.

2. Biological coating material using proteins: the principles, classification and encapsulation techniques

From the encapsulation, stand point the methods are classified according to the production process, as physical (drying), chemical (gelation) or physicochemical (coacervation and drying) treatments (Nesterenko, Alric, Violleau *et al*., 2014; Zhang, Li, Liu *et al*., 2015). The particle shape thus obtained depends on the physicochemical properties of the core, the coating material and the technique implemented (Figure 1) (Quintero *et al*., 2017).

The morphology of the capsules is divided mainly into three types (Kasapis *et al*., 2009). The capsules are characterized by presenting a solid continuous coating of the active ingredient. These capsules are generally achieved in the chemical encapsulation methods such as ionic gelation, coacervation, liposomes, inclusion and emulsification complexes. In the second type, the sphere is formed mechanically, where it is possible that some section of the encapsulated compound is exposed; among the most recognized techniques that have this type of capsules are spray-drying, extrusion, co-crystallization, and lyophilization. The third category includes more complex structures, such as multilayer microcapsule or multilayer spheres (Figure 1) (Kasapis *et al*., 2009; Kent and Doherty, 2014; Can Karaca *et al*., 2015; Zhang, Li, Liu *et al*., 2015; Zhang, Tan, Abbas *et al.,* 2015; Ashay Jain *et al*., 2016). The microparticles may exhibit from

Table 1. Properties of the microcapsules produced by different methods

Encapsulation Method	Particle size (ųm)	Max. Load (%)	Type of Process	Reference
Simple coacervation	20-200	<60	Chemical	Madene *et al.* (2006)
Complex coacervation	5-200	70-90	Chemical	Madene *et al*. (2006)
Molecular inclusion	5-50	5-10	Chemical	Madene *et al.* (2006)
Co-current spray-drying	1-100	<40	Physical	Nesterenko *et al.* (2012)
Counter-current spray-drying	50-200	10-20	Physical	Nesterenko *et al*. (2012)
Extrusion	200-2000	6-20	Physical	Madene *et al.* (2006)
Fluidized bed (drying granulation and coating)	100-200	<40	Physical	Schell and Beermann (2014)
Entrapment in liposomes	0.12 - 1	<60	Chemical	Tsai and Rizvi (2017)
Ionic gelation	0.05–1	< 60	Chemical	Fàbregas *et al*. (2013)

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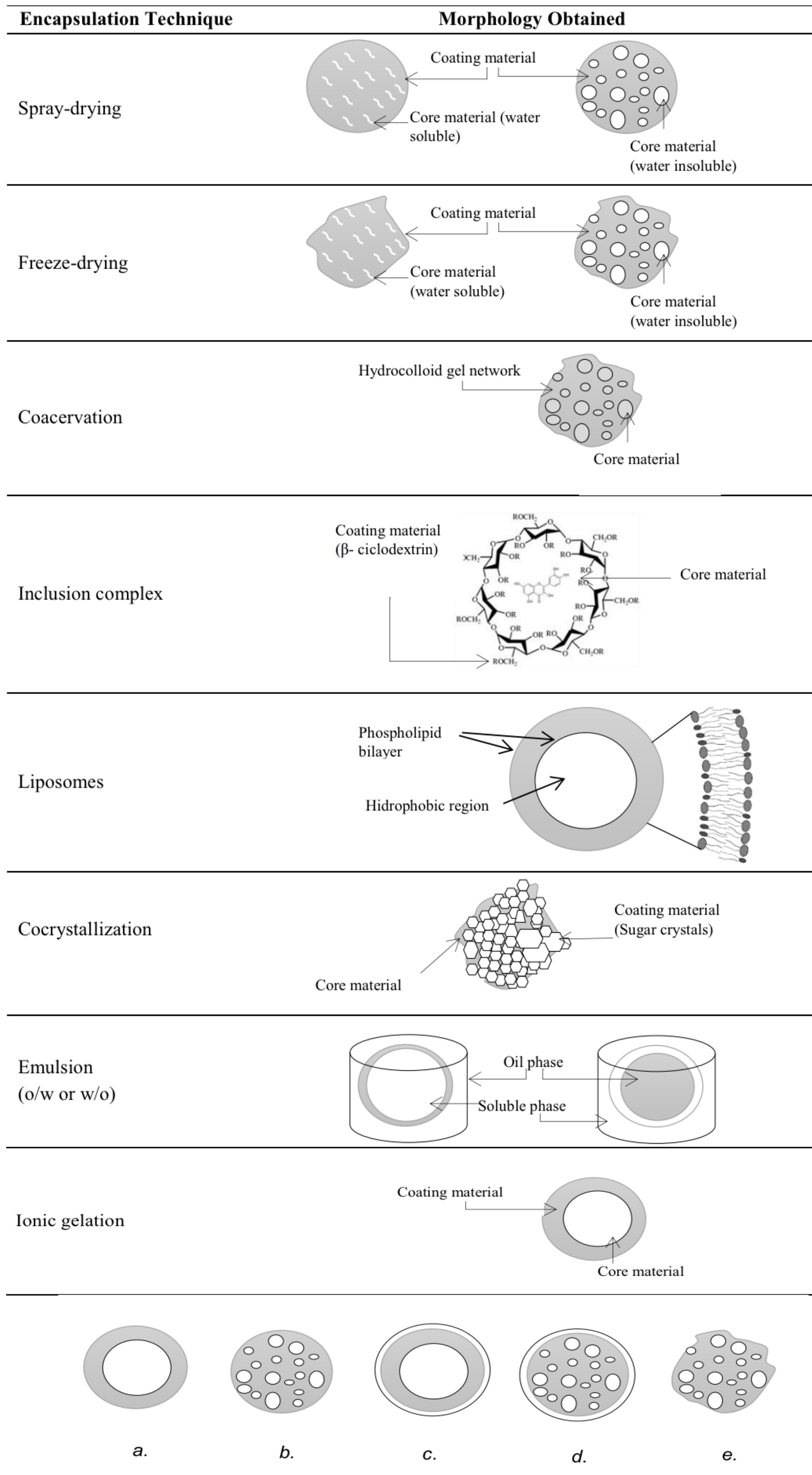

Figure 1. Types of capsules obtained according to the encapsulation technique and morphology of the particles obtained by microencapsulation: (a) single microcapsule, (b) microsphere, (c) multilayer microcapsule, (d) multilayer microspheres, (e) irregular microcapsule

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an irregular to a spherical shape. In fact, the coating material generally adapts to the contour of the particle having a great variety of shapes (Nesterenko, Alric, Silvestre *et al*., 2014). The different methods used for encapsulation and the resulting main features are present in Table 1.

Among the most popular encapsulation techniques in which proteins are used, from vegetable and animal origin, as coating materials are emulsification, spray drying, coacervation, and a technique that has great potential due to the chemical characteristics of proteins is ionic gelation (Table 2).

In the encapsulation by emulsification, the current technological development in the industry requires new emulsifiers or methodologies to obtain stable emulsions. For this reason, the search for new products to encapsulate different bioactive compounds to increase their solubility and speed is required (Burgos-Díaz et al., 2016). These colloidal systems are unstable, hence the implementation of one or more emulsifiers is needed. The emulsifiers have the ability to interact with the dispersed phase and the continuous phase, which reduces the interfacial tension and facilitate the interruption of the drops (Wang *et al.*, 2014; Zhang, Li, Liu *et al.,* 2015).

In recent years, proteins from animal and plant origin have been considered as natural emulsifiers in different industrial processes due to their highly active surface (Augustin and Hemar, 2009). Their amphiphilic property allows proteins to be strongly adsorbed at the oil-water interface, favoring the formation of emulsions. The amount adsorbed and the conformation adopted in the oil -water interface depends on factors such as the state of aggregation, the pH of the medium and the ionic strength and mainly on the amino acid composition of the protein where the adsorption occurs through hydrophobic groups present (Livney, 2010; Nesterenko, Alric, Silvestre *et al*., 2014). Among the vegetable proteins used as stabilizers in emulsion encapsulation, soy proteins, peas, beans, quinoa, pumpkin seeds, and wheat have been reported (Rayner *et al.,* 2014; Bucko *et al.*, 2015; Elsohaimy *et al*., 2015). However, it has been reported that proteins in their native state do not have the maximum emulsifying capacity; therefore, one of the most used strategies is cross-linking of the protein with a polysaccharide by means of electrostatic interactions under defined conditions of pH, concentration and ionic. This generates an encapsulation of greater solubility, greater stability, greater load of assets and longer useful lifetimes. (Jia *et al*., 2016).

The encapsulation by spray-drying is based on the formation of an stable emulsion as mention previously for the encapsulation process by emulsification. The choice of coating material is critical because it can intervene in a properties of the emulsion before drying, particle size and flow properties, as well as the retention of volatiles compounds during the process, and in the shelf-life of the powder after drying. The ideal materials for spray-drying should have a low viscosity at high concentrations, a high solubility, have a good emulsification and film forming ability, and hold efficient thermal properties (i.e., low effective diffusivity and low conductivity) in order to protect the encapsulated material during the drying process (Nesterenko, Alric, Silvestre *et al*., 2014).

Coacervation is a chemical method of phase separation, which can be classified into two types, depending on the number of polymers involved; simple or complex coacervation (Kasapis *et al.*, 2009). Complex coacervation mainly occurs by electrostatic interactions between two or more solutions of oppositely charged polymers producing two immiscible liquid phases. One is the continuous phase having a low polymer concentration, whereas, the second one is composed of the polymer-rich dense phase, also named as the coacervate phase, which in turn is used to coat a variety of active core ingredients. Usually, the coacervate complexes possess the combined functional properties of each polymer involved (Nesterenko, Alric, Silvestre *et al*., 2014). With respect to proteins derived from plant sources, coacervates obtained from soy proteins, peas, sunflower seeds and beans are reported; worth mentioning that the coacervation systems have been carried out with the protein along with polysaccharides such as alginate, carboxymethyl, cellulose, and gums (Schmitt and Turgeon, 2011; Lia *et al.*, 2015; Chang *et al.,* 2016). The polysaccharide contributes to the rigidity of the capsules and the protein contributes to the stability of the colloidal system and to the increase of the solubility of the active ingredient. On the other hand, simple coacervation only implies one polymer, and thus, it is not very popular in the food and pharmaceutical fields (Kasapis *et al.*, 2009; Tamm *et al*., 2016).

The main chemical method of encapsulation in which proteins have been used as the coating material is the coacervation method as we mentioned previously. However, ionic gelation being a chemical method has not been widely explored as a protein encapsulation technique and thus great potential.

The ionic gelation technique is also classified as external or internal. In the external gelation, a calcium salt is added to an O/W emulsion. The formation of the gel starts at the interface once the ion from the coating material is displaced by the divalent cation solubilized in

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Table 2. Encapsulation studies with plant proteins as coating materials

Coating Material	Core Material	Encapsulation Technique	Reference
Alginate/lupin protein	Linseed oil	Ionic gelation	Piornos *et al.* (2017)
Soy protein	Linseed oil	Spray-drying / Freeze-drying	González *et al.* (2016)
Soy protein isolate/sunflower protein	α-tocopherol	Spray-drying	Nesterenko *et al.* (2014b)
Soy protein isolate/pectin	Propolis	Complex coacervation	Nori *et al.* (2011)
Soy lecithin and lentil protein isolate	Canola oil	Spray-drying	Chang *et al.* (2016)
Pea protein	Linoleic acid	Spray-drying	Costa *et al.* (2015)
Pea protein	Iron	Spray-drying	de Azevedo *et al.* (2013)
Soy protein isolate/lactose blends	Fish oil	Gelation	Li and Tang (2013)
Proteins isolated from lentils and chickpeas	Linseed oil	Freeze-drying	Karaca *et al.* (2013)
Proteins isolated from lentils and chickpeas	Linseed oil	Spray-drying	Karaca *et al.* (2013)
Zein protein	Olive oil	Solvent evaporation	Mehta *et al.* (2011)
Soy protein isolate	Pepperoni oleoresin	Spray-drying	Rascón *et al.* (2011)
Soy protein isolate	Orange oil	Spray-drying	Tang and Li (2013a)
Soy protein isolate/lactose	Orange oil and flavors	Spray-drying	Tang and Li (2013b)
Soy protein isolate	Ascorbic acid/α-tocopherol/ vitamins	Spray-drying	Nesterenko *et al.* (2014b)
Soy protein isolate	Casein hydrolysate	Spray-drying	Molina *et al.* (2009)
Soy protein isolate	Paprika oleoresin	Spray-drying	Rascón *et al.* (2011)
Soy protein isolate/gelatin	Casein hydrolysate	Spray-drying	Favaro *et al.* (2010)
Soy protein isolate	Fish oil	Simple coacervation	Gan *et al.* (2008)
Soy protein isolate/gum arabic	Orange oil	Complex coacervation	Jun-xia *et al.* (2011)

water. In addition, the resulting particle size is relatively large ranging from 400 µm to 1 mm (Leong *et al.*, 2016). On the other hand, the internal gelation technique is based on the controlled release of the calcium ion in an insoluble complex and is triggered by the coating material. This is carried out by acidification of an oil system with a soluble acid causing a partition in the aqueous phase of the coating material. For instance, the aqueous phase is generally composed of alginate and calcium carbonate and then, the aqueous is added to the oil phase (composed of vegetable oil, Span® 80 and acetic acid). Usually, this technique renders particles of approximately 50 µm in diameter (Leong *et al.*, 2016).

These two processes differ mainly in their kinetic mechanism. In the external gelation mechanism, factors such as the calcium concentration and polymer composition control the sol-gel transition. Conversely, in the internal gelation the solubility and concentration of the calcium salt, concentration of the sequestering agent, and the organic acid govern the sol-gel transition (Leong *et al.*, 2016).

The coating materials used for this technique must meet two specific physico-chemical characteristics to achieve the encapsulation of components. The first feature focuses on the fact that the coating material has the capacity to form ionic bonds with polyication and material has the capacity to form gels. The most popular material for the process of encapsulation by the ionic gelation is sodium alginate. Alginates are a family of linear polysaccharides, containing varying amounts of β-D-mannuronic acid and α-L-guluronic acid. Their composition (given by the characteristic mannuronic/ guluronic relationship) and sequences varies depending on their source. Salts of alginic acid, such as sodium alginate, are composed by three blocks, a block having of only mannuronic acid, blocks of only guluronic acid and a block where the two acids are present. When two chains of the guluronic acid block are aligned, coordination sites are formed. The shape of the loops of these chains, form cavities between them that are sized to accommodate the calcium ion and are also coated with carboxylic groups and other electronegative oxygen atoms. After the addition of calcium ions, the alginate undergoes conformational changes, giving rise to the well-known alginate gelling model known as the "egg box". This is based on the dimerization of the chain and model, subsequently, on the greater aggregation of the dimers (Miyazaki *et al.*, 2000).

In the case of proteins, they for this type of

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encapsulation technique due to their ability to form gels by structural modifications, and to the presence of some amino acids in their structure.

This is the case of ionizable amino acids such as arginine, aspartic acid, glutamic acid, cysteine, histidine, lysine and tyrosine, that favor the emulsifying capacity of the proteins and the capacity formation of gels; the ability to generate protein-protein bonds or in the case of encapsulation by ionic gelation; protein - cation - protein bonds. furthermore, among these side groups are the thiol groups, specifically from the amino acids cysteine and methionine, that favor the formation and the strengths of the gels. Asparagine, glutamine, serine, and threonine contain non-ionic polar groups, which are often found on the surface of protein, allowing for a strong interaction with the aqueous medium (Nedovic and Willaert, 2013).

It is noteworthy the amino acids such as aspartic acid (Asp, D) and glutamic acid (Glu, E), have the ability to react with calcium ions, forming aggregates that potentiate gelation. These two amino acids have a carboxyl group, which presents a chemical similarity with sodium alginate. Thus, a large presence of these two amino acids in the structure of protein favors the interaction with the calcium ions and in turn the encapsulation of assets. In reports of aminograms it has been found that these two amino acids are found in a greater proportion of vegetable proteins (i.e. soy, sunflower, rice, and peas) than in animal proteins; highlighting the plant source as a source of potential coating material for this encapsulation technique. On the other hand, the gelling of proteins is based on the capacity of self-folding capability , forming spontaneous networks that are expressed in the formation of gels (Augustin and Hemar, 2009). Heat treatment is a common gelling strategy, which includes the cold and hot gel sets. The cold gelation of proteins is performed by heating the proteins in the solution, followed by a cooling step, and a final addition of the active ingredient (Tavares *et al*., 2014). Conversely, in the heated gelation proteins interact with the core material first and then undergo a heat treatment where the polypeptide chains are displayed by exposing the functional groups, which in turn, interact with the core material, forming a three-dimensional network (Tavares *et al.,* 2014). Another strategy involves a two-step protein gelling and thus it is called acid gelling. First, the pH of the solution is adjusted above the isoelectric point (pI) of the proteins to prevent their gelation. Then, the pH of the solution is adjusted to a value close to the pI, where the electrostatic interactions within the protein molecules are improved forming particulate gels (Augustin and Hemar, 2009).

3. Structural modifications in vegetable proteins: A reasonable strategy to improve their encapsulating capability

In principle, any protein capable of forming a film has a potential capability for encapsulation, provided that the film is also formed on the surface of the core material, e.g., oil droplets (Gharsallaoui *et al.*, 2012). The process of film formation is based on the dynamic structure of the protein and its ability to self-associate. This phenomenon could be triggered by different singularities such as solvent conditions (i.e., polarity, pH changes, and presence of electrolytes), thermal treatment, or solvent removal.

Water, ethanol, and acetone are the solvents used to prepare protein-based film-forming solutions. However, the dispersion of proteins in solvents requires the addition of reducing agents such as mercaptoethanol, sodium borohydride or sodium sulfite; the adjustment of the pH and the addition of salts to adjust the ionic strength of the solution. In addition to the reducing agents and the adjustment of the conditions of the media, the dispersion of proteins, in most cases, need to generate an unfolding of the structure that having the formation of molecular interactions that generate the film. In most cases, it is achieved with thermal treatments (Gharsallaoui *et al.*, 2012).

Even though proteins are versatile materials with interesting properties for encapsulation, it may be necessary to modify the inherent properties such as solubility, hydrophobicity, hydrophilicity, the gelling, emulsifying, foaming and the surfactant properties. As a result, they become a coating material with adjusted characteristics according to the properties of the active compound, the ligation system having higher encapsulation efficiencies (Nedovic and Willaert, 2013; Zhang, Tan, Abbas *et al.,* 2014; Nesterenko, Alric, Violleau *et al.*, 2014). Several modifications can be conducted in proteins since amino acids have side chains of different sizes, shapes, charges and chemical reactivity.

The term "crosslinking" is commonly used to describe the intra or inter covalent bonding of a protein. As a result, the molecular size and shape, and the functional properties may be affected by crosslinking. Different methods can be used for crosslinking purposes, ranging from physical to enzymatic and chemical modifications (Jancurová *et al*., 2009; Nesterenko, Alric, Silvestre *et al*., 2014). Some of the feasible modifications of proteins are illustrated in Figure 2 (Quintero *et al.,* 2017).

The structural modifications of the proteins by

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physical treatments are classified mainly into two types: (i) having a change of pressure or (ii) having a thermal treatments. Both treatments generate unfolding of the tertiary structure of the protein, however, the most widely used treatments are the thermal treatments, looking for a partial unfolding of the protein or reaching its denaturation. The main disadvantage of the thermal treatment is its lack of uniformity, since as the reaction progresses, breaking of covalent bonds, hydrophobic interactions, hydrogen bridges and disulfide bridges, alters the structure but the secondary structure remains unchanged. Once the chains are fully deployed, the denaturing process is completed, which is sometimes not desirable. The magnitude of these changes depends on the source and environmental conditions such as pH, solvent, the presence of salts, surfactants, and concentration of the protein (Nesterenko *et al*., 2013).

Another characteristic that can be modified in the structure of the vegetable source protein and potentiate its encapsulating capacity is its size of it. The main technique used to modify the size of proteins is hydrolysis, that can be it chemical, physical or enzymatic. Enzymatic hydrolysis occurs due to the breakdown of the peptide bonds of the protein caused by a proteolytic enzyme such as transglutaminase, peroxidase, lipoxygenase and catechol oxidase (Nedovic and Willaert, 2013). When the protein decreases its molecular weight by enzymatic hydrolysis, it has the ability to be more flexible and generate more interactions with the medium, since breaking of the peptide bonds exposes the amino and carboxyl groups. This favors the proteins and allows them for entering the interface of the colloidal systems of the emulsions, increasing the stability of the system (Felix *et al*., 2016). In the case of gelation, the hydrolysed protein favors the formation of gels by the interactions groups exposed by the treatment; however, an excessive hydrolysis generates peptides leading to the loss of the functionality of the protein as a coating material, but sometimes potentiate biological activities such as the antioxidant, antimicrobial or larvicide functionality (Felix *et al.*, 2016).

On the other hand, the chemical modifications in the structures of the proteins have been the most relevant modifications in the last decade. The reactions applied for structural modifications are deamidation reactions, acylation, chemical hydrolysis and cationization reactions (Nedovic and Willaert, 2013). All these reactions modify the secondary structure of proteins through the use of different compounds that could form a branch of the biopolymer, cause an aggregation of hydrophobic compounds, form a charged biopolymers (cationic or anionic) or a biopolymer in which certain specific amino acids are exposed to react with other components of the medium. In order to understand the chemical nature of protein modifications and the resulting changes in the properties a detailed description of the fundamentals of the main chemical modifications is reported as follows:

3.1 Maillard Reaction

In recent years, a great number of researchers have conjugated proteins with polysaccharides using the Maillard reactions. In general, this method efficiently improves the emulsifying properties, solubility,

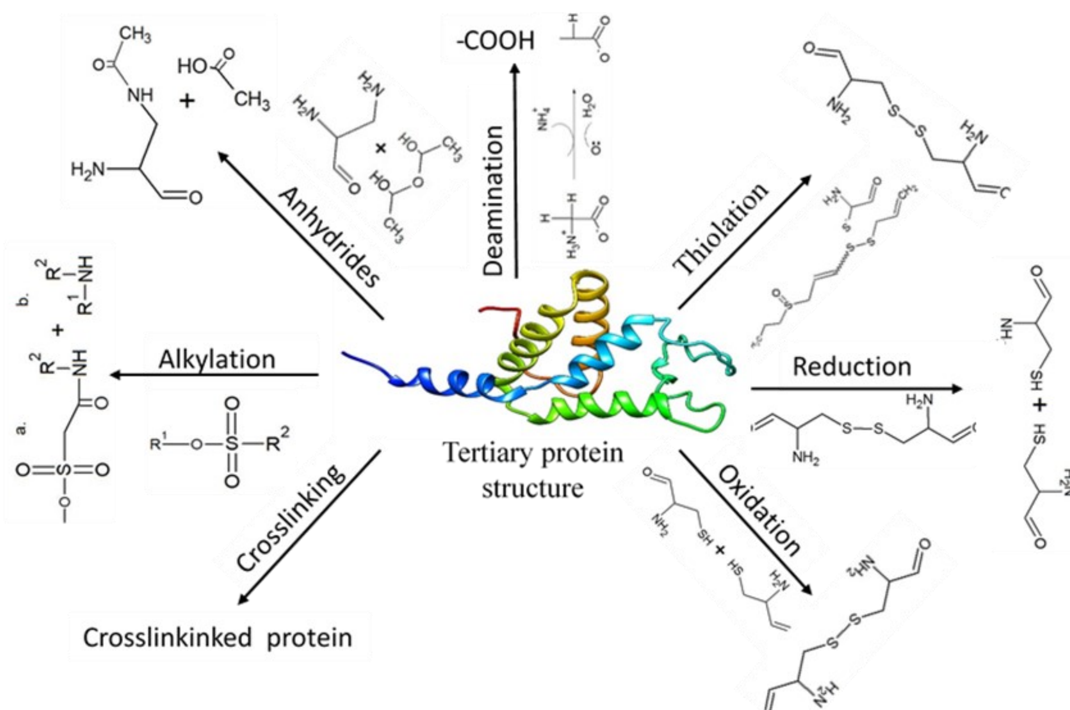

Figure 2. Schematic for the chemical modifications of proteins

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antibacterial and antioxidant effects and reduces the allergenicity of proteins (Lia *et al*., 2015). The Maillard reaction occurs naturally between a reducing sugar and an amino acid. The reaction mechanism implies an initial condensation step between the reducing end group of a carbohydrate and an amino acid. These products are then isomerized to form the typical Amadori rearrangement of compounds including a Schiff base. Thereafter, the protein-carbohydrate conjugate fragments create reactive intermediates and could form partially crosslinked protein structures, resulting in a greater degree of crosslinking of insoluble polymeric compounds (Figure 3a) (Evans *et al*., 2013; Zhang *et al.,* 2014).

3.3 Acylation reaction

The acylation reaction is performed to covalently attach a fatty chain to a protein. In this scenario, three functional groups of proteins are capable of creating a covalent bond with a fatty acid derivative:

i. Cysteine thiol groups leading to the formation of thioester bonds,

ii. Hydroxyl groups of serine and the amine group of lysine leading to the formation of an ester bond,

iii. The ends of polypeptide chains that form amide bonds (Nesterenko, Alric, Silvestre *et al*., 2014).

Conventionally, the acylation of a protein in an aqueous alkaline medium is carried out by the Schotten-Baumann condensation reaction (Nesterenko, Alric, Silvestre *et al*., 2014). Under these conditions, the amine groups react primarily with the fatty acids. Thus, in a protein, the free amine is represented by the terminal end of the chains and lysine residues (Figure 3b).

The functionality of proteins depends on their surface activity. In an aqueous medium, the protein structure is rearranged so that the hydrophobic groups locate themselves in the interior core, whereas the hydrophilic groups remain on the outside of the structure. The goal of the acylation is to introduce new hydrophobic groups on the surface of the protein and thus have a significant influence on the primary solubilizing, gelling and emulsifying properties (Nesterenko *et al.,* 2013).

3.3 Cationization reaction

The cationization reaction implies the generation of positive charges on the molecules by grafting cationic groups (Nesterenko, Alric, Silvestre *et al*., 2014). This functionalization could change the isoelectric point of the proteins and therefore, their solubility as a function of pH and the affinity towards the anionic substance as well (Nesterenko, Alric, Silvestre *et al*., 2014). The most widely studied cationic reagent has a quaternary ammonium function (Nesterenko, Alric, Violleau *et al*., 2014). Theoretically, the cationization could be conducted on natural polymers such as proteins and polysaccharides (Figure 3c), since they can react with a molecule having a cationic quaternary ammonium function (Nesterenko, Alric, Violleau *et al*., 2014).

In general, proteins have two major groups such as the amino and primary carboxyl groups, respectively. At a basic pH, the nucleophilic reactivity of the amino groups is greater than that of the carboxyl groups. Therefore, the cationization reaction is carried out mainly in the amine groups. Currently, the cation-modification of animal proteins is used to improve their solubility, antibacterial and hydrophilic properties (e.g., water absorption and swelling abilities) (Nesterenko, Alric, Silvestre *et al*., 2014).

Among the applications of structural chemical modifications in vegetable proteins, encapsulations with proteins isolated from legumes (e.g., soybeans, peas, chickpeas and lentils), sunflower seeds and cereals (e.g., oats, wheat, barley and corn) (Nedovic and Willaert, 2013; Gaonkar *et al*., 2014; Carbonaro *et al*., 2014; Nesterenko, Alric, Silvestre *et al*., 2014; Dong *et al*., 2015; Ashay Jain *et al.,* 2016). In these cases, the acylation reactions, cationization, chemical hydrolysis have been applied, and Maillard reactions. Achieving an increase in encapsulation efficiency from 82.6% to 94.8% in the case of soybeans and from 79.7% to 99.5% in the case of sunflower seed proteins treated by acylation. They concluded that the structural modification of these proteins increased the affinity between the active ingredient and the coating material, improving the encapsulation process, the hydration and the net charge of the protein (Nedovic and Willaert, 2013; Nesterenko, Alric, Silvestre *et al*., 2014; Zhang, Tan, Abbas *et al.,* 2014; Carbonaro *et al.,* 2014; Teng *et al*., 2015).

Currently, most studies on plant proteins involve spray-drying and coacervation as preferred encapsulation methods. However, as emerging vegetable proteins include pumpkin seeds (*Cucurbita maxima*), ahuyama (*Cucurbita moschata*), quinoa (*Chenopodium quinoa*) and chia (*Hispanic Salvia*), and legumes such as black bean (*Phaseolus Vulgaris*), lentils (*Lens culinaris*), peas (*Pisumsativum*) and chickpeas (*Cicer arietinum*). All these sources are considered as vegetable sources that have a high protein content, ranging from 24% to 36%, 25% to 30%, 12% to 23%, 17% to 22%, 22% to 26%, 12% to 23% , 10% to 18% and 15% to 25%, respectively (Quanhong and Caili, 2005; Aluko *et al*., 2015; Ariyarathna and Karunaratne, 2015; Bucko *et al*., 2015;

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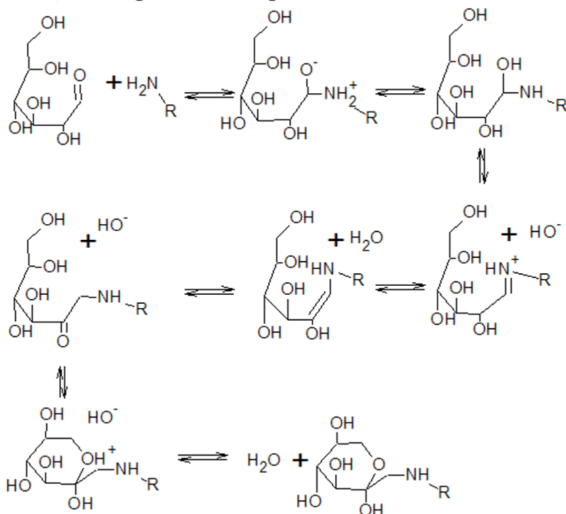
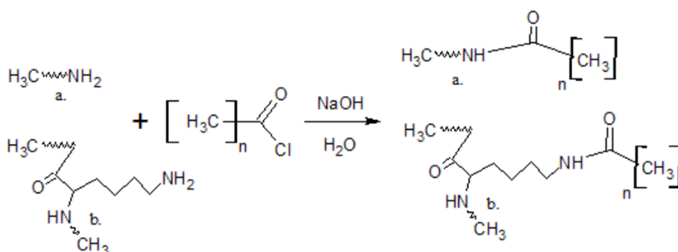
1° The carbonyl group of the sugar reacts with the amino group of the amino acid, rendering N-substituted glucosamine and water.

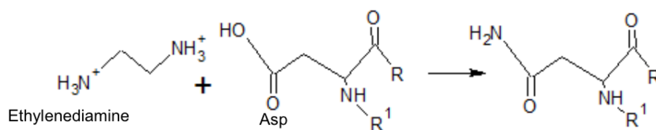


2° The unstable glucosamine is then subjected to the Amadori reaction producing ketosamines and water.

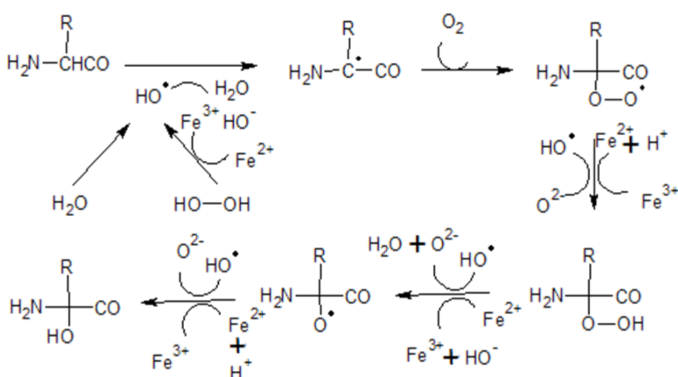
A. The mechanism of a Maillard reaction



B. Schotten-Baumann N-acylation reaction (a) In a C-terminal end and (b) In a lysine residue



C. Ethylene diamine-induced cationization with aspartic acid



D. Oxygen-free and radical-mediated oxidation of proteins

Figure 3. Mechanism of the reaction of some structural modifications used in proteins: (A) Maillard reaction, (B) Acylation reaction, (C) Cationization reaction, (D) Oxidation -reduction reactions.

Nongonierma *et al*., 2015; Shevkani *et al*., 2015; Torres-fuentes *et al*., 2015; Wang *et al*., 2015; Xu *et al*., 2016).

Further, proteins both in native and modified form isolated from the seeds of chia, pumpkin, ahuyama, quinoa, and black beans could stabilize emulsions. Moreover, the antioxidant activity and nutritional content of some peptides modified by chemical and enzymatic hydrolyses, have been evaluated assessing the contribution of essential amino acids on those properties (Quanhong and Caili, 2005; Aluko et al., 2015; Ariyarathna and Karunaratne, 2015; Bucko *et al.,* 2015; Nongonierma *et al.,* 2015; Shevkani *et al.,* 2015; Torres-fuentes *et al.*, 2015; Wang *et al.*, 2015; Xu *et al.,* 2016). This opens a door of opportunities to study the functional properties and encapsulation capacity of different vegetable sources of proteins.

4. Conclusion

Vegetable proteins and their possible role in the encapsulation process as a coating material point out the high usefulness and versatility of these macromolecules. The conventional encapsulation systems described previously often involve relatively expensive proteins such as albumin with wide range pharmaceutical applications. The use of plant proteins such as wheat gluten, soy protein and other vegetable sources as encapsulation agents is still unexplored. Plant proteins are abundant, versatile, relatively inexpensive, biodegradable and exhibit good emulsifying properties. Further, they have functional groups which are available for physical, chemical or enzymatic modifications. As a result, a better-controlled release system and a higher encapsulation efficiency and coating efficiency are achieved by using mainly spray-drying, coacervation and in a lower degree by ionic gelation. Further, these modifications also played a major role in the stability, porosity and release properties of protein-based microspheres or microcapsules, and can be adjusted by the proper selection of treatment/reagent, time, and protein/reagent ratio.

Conflict of Interest

The authors declare no competing financial interest.

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