Food Biopolymers: Structural, Functional, and Nutraceutical Properties: Food Proteins: An Overview

Nisar Ahmad Mir, Mamta Bharadwaj, Basharat Yousuf, Khalid Gul, Charanjit Singh Riar, and Sukhcharan Singh

Introduction

Proteins are considered as the most abundant organic molecules in living systems and are usually required in large quantities because they are the building blocks of the body therefore a way more diverse in structure and function than any other class of macromolecules. All proteins are made up of one or more chains of amino acids which share a general structure consisting of a central carbon atom, also known as alpha (α) carbon, bonded to an amino group (NH_2), a carboxylic group (COOH), and a hydrogen atom (Fig. 1). Each amino acid has another atom or group of atoms bonded to the central atom known as R group which determines the identity of the amino acid. For instance, if R group is a hydrogen atom, then the amino acid is glycine and if it is methyl (CH_3) the amino acid is alanine. Food quality is usually determined by its nutritional and functional properties which primarily depend upon the type of proteins present in the food material. Nutritional properties of proteins include biological value, protein efficiency ratio; protein digestibility corrected amino acid score, nutritional index, and corrected amino acid score (Mir et al. 2018). These nutritional properties depend upon the type of amino acids and their bioavailability or in other words properties affecting the body after passage of food

N. A. Mir · M. Bharadwaj · C. S. Riar · S. Singh
Department of Food Engineering and Technology, SLIET, Sangrur, Punjab, India

B. Yousuf
Department of Post-Harvest Engineering and Technology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, India

K. Gul (✉)
Department of Food Process Engineering, National Institute of Technology, Rourkela, Odisha, India

Department of Food Science and Engineering, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China

A. Gani, B. A. Ashwar (eds.), *Food biopolymers: Structural, functional and nutraceutical properties*, https://doi.org/10.1007/978-3-030-27061-2_9

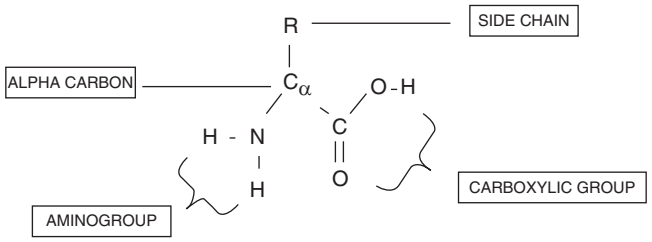

Fig. 1 General structure of proteins

into the alimentary canal. In addition to this the effectiveness of use of proteins largely depends upon the functional characteristics which can be tailored to meet the needs of food products and manufacturers. The functional attributes of the food proteins depend primarily on their molecular size, charge distribution and three dimensional structures. Among functional properties the most important ones include hydration, water/oil combination, gelling, emulsification, foam formation and rheological behaviors. Therefore, proteins in the form of isolates or concentrates can be used in a wide variety of bakery, meat, dairy and other food products in order to impart desirable sensorial and textural characteristics and to produce some novel functionalities.

Protein Structure

Protein structure sets the foundation for its interaction with other molecules in the body and therefore determines its function. Protein molecules are usually single, unbranched chains of amino acids and monomers. In general protein molecules are made up of 20 different amino acids (Fig. 2). The amino acid side chains in a peptide can become modified and in turn extend the functional repertoire of amino acids to more than hundred different amino acids. In addition to this the amino acid sequence of proteins determine its three dimensional structure (confirmation) which in turn determines the functionality of the protein.

In general a protein structure is divided into four different types depending upon the sequence of amino acids present in the structure. These four levels of structure determines the shape of proteins, the four types of structure are:

1. Primary structure

   The primary structure of proteins consists of a sequence of amino acids linked together by peptide bonds and includes any disulfide bonds. It is also characterized by genetic code and post translational covalent modifications and it ultimately determines the physicochemical and functional properties of food. However the secondary, tertiary and quaternary structure determines the biological functions of proteins.
2. Secondary structure

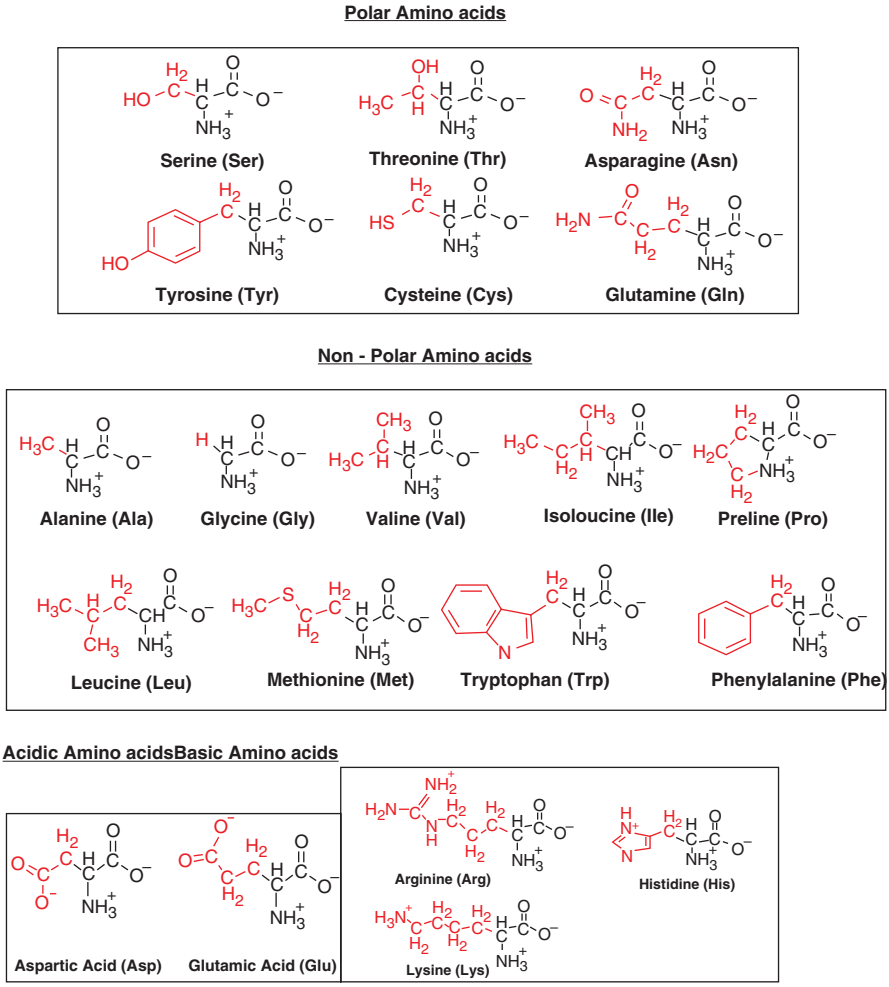

Fig. 2 Structure of twenty different amino acids

The secondary structure comprises of localized parts of a polypeptide chain (e.g. the α-helix or β-sheet with hydrogen bonds as their backbone. These special structures comprise the secondary structure of protein molecules. Secondary structure is usually due to aperiodic and periodic structures. Random coil is an example of aperiodic structure, whereas, helical and extended are examples of periodic structure. Stability of these structures are provided by decrease in local free energy by the rotation of φ and ψ angles, local non-covalent interactions between amino acid side chains, as well as hydrogen bonding between CO and N-H groups (Nelson and Cox 2013; Voet et al. 2013).

(a). *Alpha-helix proteins*: The secondary structure of some proteins is essentially all alpha-helices. Myoglobin is a good example of this type. Damodaran

(2008) proposed that α-helical structure is associated with the amphiphilic nature due to the presence of helix surface being made up of hydrophobic (non polar residues) as well as hydrophyllic (polar) residues. The nonpolar residues may also position themselves in the hydrophobic interior of the protein molecule. The α-helixis sometimes also termed the 3.6_{13}-helix, due to the 13 backbone atoms in the hydrogen bonded loop in the structure of this molecule. It should be noted that out of 20 amino acids, the cyclic imino acid proline cannot form α- helices which is because of its ring structure the N-Cα bond is unable to twist and the φ angle remains fixed at 70° due to this reason it is also known as 'α helix breaker' which is because of its ring structure. It should also be noted that of the 20 amino acids commonly found in foods, only proline is likely to adopt the cis-isomer (Damodaran 2008; Nelson and Cox 2013).

(b). *Beta-sheet proteins*: Some proteins are largely formed from beta—sheets and include some antibodies and T-cell receptors. There are two types of β-pleated sheet structures, parallel β-sheet or antiparallel β-sheet depending on the direction of the polypeptide strands. In parallel β-sheet the N → C strands run parallel, whereas in the antiparallel β-sheet N → C strands run in opposite direction. In the antiparallelβ-sheet structure hydrogen bonds form a straight line which provides additional stability to the structure making antiparallel β-sheet more stable than its parallel β-sheet counterpart. In the parallel β-sheet structure hydrogen bonds are formed at an angle, thus the stability of the hydrogen bonds, and therefore the stability of the structure are reduced. Also, generally, β-sheet is more stable than the α-helix structure; therefore, proteins with large segments of β-sheet structures are likely to be more heat stable or have higher denaturation temperatures (Nelson and Cox 2013; Voet et al. 2013).

(c). *Alpha-helix and beta sheet proteins*: Some proteins contain both alpha helix and beta sheets. Hexokinase is an example of alpha/beta structure

3. Tertiary structure
   Tertiary structure of the protein includes the total three dimensional arrangement of the polypeptide chain comprising of hydrophobic interactions, hydrogen bonds (non-covalent bonds in general) and sulfur-bridges. It also includes simple dimmers to homo-oligomers and complexes with defined or variable numbers of subunits. The tertiary structure of proteins depends largely on the sequence of amino acids in a polypeptide and is stabilized through hydrogen bonding between CO and NH groups e.g. heamoglobin.
4. Quaternary structure
   It is the association of two or more polypeptides into a multi-subunit complex. Heamoglobin is one example of quaternary structure another example is collagen which is a widespread connective tissue protein and consists of three polypeptide chains. It has been observed that proteins having molecular weight greater than 100 kDa are likely to have more than one polypeptide and more likely to have quaternary structures. Many food proteins having quaternary structures include

cereal and soy proteins, with different polypeptide subunits, bovine milk β-lactoglobulin which is interesting because it is a monomer of 18 kDa at pH >8 and its structure forms a dimer at pH 5–8 and an octamer at pH 3–5 (Damodaran 2008).

All the four structures of protein molecules are shown in the Fig. 3

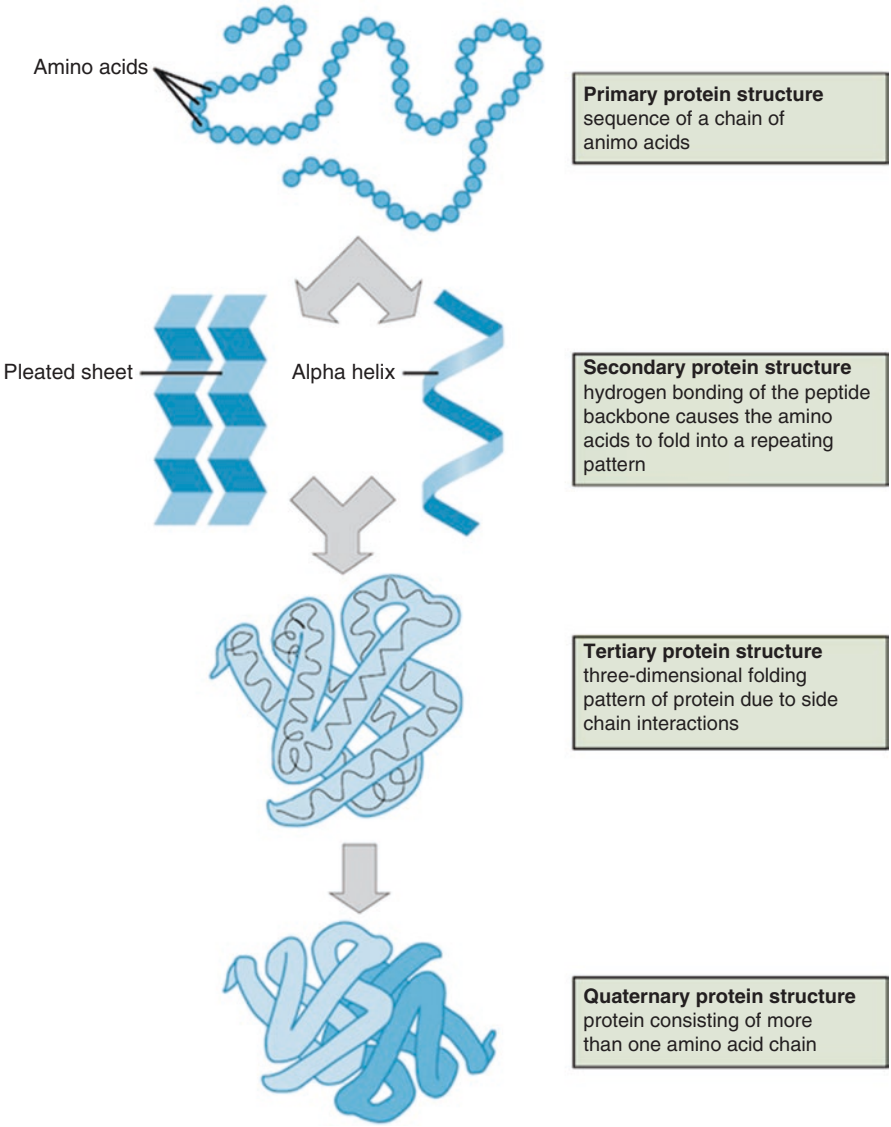

Fig. 3 Primary, secondary, tertiary and quaternary structure of protein molecules

Favorable Interactions in Protein Molecules

The native structure of protein has low energy than the denatured state of the protein. The denaturation of protein is therefore the consequence of breaking labile (non-covalent) bonds that maintain this lower energy of native state. Several non-covalent forces that are responsible for stabilizing the structure of protein are the van der Waals interactions, hydrogen bonding and hydrophobic effect. However these forces are opposed by major destabilizing force, which is associated with conformational entropy loss upon protein folding. Other forces, such as electrostatic interactions, can be either favorable or unfavorable, depending on the context. Nevertheless, the backbone of protein structure is stabilized by covalent bonds (disulfide bonds), however non covalent interactions are required to maintain secondary, tertiary and quaternary structure of proteins molecules.

Non-Covalent Bonds

Hydrophobic Interactions

Regarding hydrophobic interactions they are not attractive in nature but result from the inability of water to form hydrogen bonds with certain side chains. These interactions are the main forces that drive protein folding and are hence important in determining the native structure of protein. Thermodynamically they are unfavorable interactions of protein molecules with water, thus minimize their association with water. Hydropathies are used to describe the hydrophobic and hydrophilic tendencies of each amino acid residue, greater the hydropathy of an amino acid residue, the more likely it will orient orbury itself to the interior of the protein molecule.

Electrostatic Interactions

Electrostatic interactions like van der Waals forces may be attractive or repulsive in nature resulting from induced dipole which is due to the polarization of electron cloud between neutral atoms in protein molecules. However, these forces are relatively weak, the strength of these forces decrease rapidly with increase in distance.

Hydrogen bonds are formed by sharing of a proton between donor and acceptor groups. The strength of hydrogen bond is 2–5 kcal/mol and the ideal distance is 2.8–3 Å. Usually these bonds involve the interaction of hydrogen atom, which is covalently attached to an electronegative atom such as O, N and S, with a second electronegative atom. The most common types of hydrogen bonding include bonding between N–H and C=H groups in α-helix and β-sheet structure of protein.

Ionic interactions are actually salt bridges between ionizable groups of a protein that has negative and positive charge. Damodaran (2008), Li-Chan (2012) proposed that electrostatic interactions between oppositely charged ion pairs are strong, and certainly have an influence on protein folding patterns, therefore they contribute little to the stability of a protein since these charged groups can also interact with water.

Covalent Bonds

Disulfide bonds (S–S) are the only covalent cross linkages found in protein molecules and are formed between sulfhydryl (thiol) groups of two cysteine molecules in the presence of oxidizing environment. Disulfide bonds can be inter or intramolecular and help in the stabilization of folded protein structure.

In general the stability of protein structure is the result of covalent and noncovalent interactions. Table 1 presents the energy of the forces which are involved in the stability of protein structure.

Classification of Proteins

All proteins are remarkably similar in structure because they contain amino acids. As of now little is known about their structure so classification based on this criterion is not completely possible. However various criteria's are used for the classification of proteins which are as under:

a. Classification based on the source of protein molecule

- Animal proteins usually derived from animal sources like meat, milk, egg and fish and usually are higher in quality because they contain all the essential amino acids.
- Plant proteins also known as low quality proteins since they contain low content (limiting amount) of one or more of the essential amino acids.

b. Classification based on the shape of protein molecule

Table 1 Adapted and modified from Li-Chan (2012)

S. No.	Type of molecular forces involved	Energy (kJ/mol)
1	Covalent bonds	330–380
2	Electrostatic interactions	42–84
3	Hydrogen bonds	8–40
4	Hydrophobic interactions	4–12
5	Van der Waals	1–9

- Globular or Corpuscular proteins (e.g. Cytochrome C, Blood proteins, Enzymes, nutrient proteins)
- Fibrous or Fibrillar proteins and they can be further classified as collagen, elastin, keratin and fibrion

c. Classification based on composition and solubility

- Simple proteins or holoproteins, they can be further classified mainly on the basis of their solubility like protamines and histones, albumins, globulins, glutelins, prolamines, scleroproteins and albuminoids.
- Conjugated or complex proteins or heteroproteins can be further classified based on the nature of the prosthetic group present. The various divisions are metalloproteins, chromoproteins, glycoproteins, phosphoproteins, lipoproteins and nucleoproteins. (Instead of metalloproteins, chromoproteins etc., the terms metalloproteids, chromoproteids etc., are sometimes used.)
- Derived proteins which are derivatives of proteins resulting from the action of heat, enzymes or chemical agents. They are further classified as primary derived proteins and secondary derived proteins. Primary derived proteins include proteans, metaproteans or infra proteins and coagulated proteins. Secondary derived proteins include proteoses, peptones and polypeptides.

d. Classification based on biological function

- Depending upon their physical and chemical structure and location inside the cell, different proteins perform various functions. Because of their diverse nature proteins may be catgorised under following groups which is based on the metabolic functions they perform and include enzymatic proteins, structural proteins, transport or carrier proteins, nutrient and storage proteins, contractile or motile proteins, defense proteins, regulatory proteins and toxic proteins.

In addition to this Osborne (1924) classified proteins into four groups on the basis of their extraction and solubility in water (albumins), dilute saline (globulins), alcohol hater mixtures (prolamins), and dilute acid or alkali (glutelins). The major seed storage proteins include albumins, globulins, and prolamins. According to this definition, albumins are soluble in water, but globulins are insoluble in water and soluble in dilute salt solutions. Prolamins are alcohol soluble and glutelins are alkali soluble proteins. Albumins and globulins are referred as soluble proteins (Salunkhe et al. 1992). The general classification of proteins is shown in Fig. 4.

Main Storage Proteins of Cereals

It is important to pay attention towards the nature of protein before employing them in any process. Usually they fall into two classes: the prolamins, which are present in cereals and a predominance of globulins with some albumins in pseudo-cereals

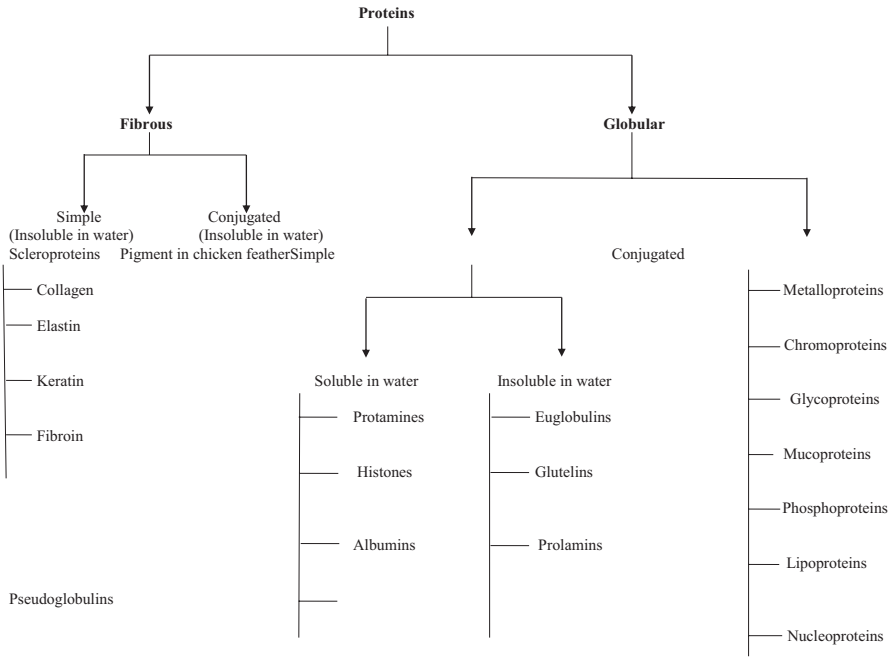

Fig. 4 General classification of proteins (adapted and modified from Voet et al. 2013)

(Shewry et al. 2002). The storage proteins belong to different groups and have significantly different structures and properties. Globulins the major storage proteins present in pseudo-cereals may be classified into groups depending on their sedimentation coefficients (which reflect their molecular masses). Typical 11S globulins are hexameric with a molecular weight of the order of 250–400 k. The subunits consist of two chains which are acidic and basic and linked by single disulphide bond. 7S globulins are trimeric and have molecular weights in the order of 150–190 k and have no disulphide bonds. Regarding albumins, they have a molecular weight of 8–15 k and contain a small and large subunit, which are linked by two disulphide bonds. Taylor et al. (2016) reported that the major storage proteins of pseudo-cereals are similar to the legume proteins, they contain 2S albumin and 11S globulin storage proteins, with 7S globulin present in buckwheat and amaranth. Tandang-Silvas et al. (2012) found that globulins from pseudo-cereals have predominantly β-sheet structure with β-barrel confirmation and therefore they are associated in the formation of good quality doughs. It has also been found that the 11S type globulins of rice oats and pseudo-cereals polymerize by disulphide bonding. Whilst the composition and structure of these storage proteins share some similarities with glutenin, but there are some important differences in terms of aminoacid composition, sequence and secondary, tertiary and quaternary structure.

Functional Properties of Proteins

The functional properties arise from a number of physical and chemical properties and affect the behavior of proteins in food systems during processing, cooking, storage and consumption. In addition to this they are also influenced by other factors such as pH, temperature, radiation or the presence of ions in foods. The functional properties of proteins play an important role as they determine the applications of particular type of protein in different systems. As food systems are usually complex therefore selecting specific type of protein for a particular application will depend upon its functionality. The functional properties depend upon the type of amino acids and functional groups present in the particular amino acid and also depend upon the interactions which are responsible for stabilizing the native structure of protein molecule. The favorable interactions in protein molecules may be covalent, hydrophobic, electrostatic, hydrogen bonds and ionic interactions. These interactions also determine the type of functionality of a protein in different food systems.

In general, several factors affect the functional properties of food proteins, namely intrinsic factors such as amino acid sequence and composition, secondary and tertiary structures, hydrophilic/hydrophobic character of the protein surface, net charge and charge distribution and molecular rigidity/flexibility of the protein and extrinsic factors such as pH, ionic strength, temperature and interactions with other food components (Zhu and Damodaran 1994). It is important to note that processing of foods may lead to structural modification of the native structure of the protein reversibly (unfolding) or irreversibly (denaturation) depending upon the processing conditions and technologies applied. Food, chemical and pharmaceutical industries rely upon these functional properties of proteins with the aim of improving the stability of the formulations or developing novel foods. Some functional of proteins which are important from the technological point of view are discussed below.

Solubility

Solubility is one of the most important properties of proteins since other functional properties like emulsion activity, emulsion stability, water binding capacity, oil binding capacity, foam capacity and foam stability are directly related to solubility (Stefanović et al. 2017). Some researchers have even concluded that solubility is the prerequisite for other functional properties. Solubility is also considered as the most important applicable scale for denaturation and aggregation thus it is a good indicator of protein function. A number of factors which play a predominant role in solubility are amino acid composition and number of hydrophilic groups present in the particular amino acid and the pH. Protein surface has a net charge that depends on the number and identity of the charged amino acids, and also depends upon the pH. For example at a specific pH the positive and negative charges will be balanced and the net charge will be zero this pH is called the iso-electric point. Most of the food proteins have iso-electric pH ranging from 3.5 to 4.5. A protein molecule has

lowest solubility at its iso-electric point so if there is a charge at the protein surface, the protein prefers to interact with water, rather than with other protein molecules, thus this charge makes it more soluble. The surfaces of proteins are occupied by amino acid residues that interact with water these amino acids are referred to as hydrophilic amino acids and include arginine, lysine, aspartic acid, and glutamic acid. At pH 7 the side chains of these amino acids carry charges positive for arginine and lysine, negative for aspartic acid and glutamic acid. As the pH increases, lysine and arginine begin to lose their positive charge, and at pHs greater than 12 they are mainly neutral. On the other hand when pH decreases, aspartic acid and glutamic acid begin to lose their negative charge and it has been found that at pH less than 4 they are mainly neutral.

Gel Forming Properties

It is an important functional attribute of proteins which is related to food processing. Many foods are in the form of gels and the main structural building element in such type of foods is protein. In addition to proteins, pectin, starches and gums are also associated in the formation of strong gels. The process of gelation is a basic fundamental for various types of food systems like milk gels, comminuted meat and fish products, other meat products, cake fillings, fruit jellies, bread dough's and others. It is the main criterion which is frequently used to evaluate quality of proteins. Many quality characteristics like adhesiveness, gumminess, juiciness and other textural properties are directly related to the gelling properties of proteins. Visco-elastic properties of many foods are also related to gelation properties of food proteins. The gelation properties also affect other functional properties of proteins like water binding capacity, oil binding capacity, emulsifying capacity, emulsion stability etc. It also plays a major role in stabilizing various types of emulsions and foams. Protein gels can be formed by employing different type of approaches like heating, enzymatic process, heating in combination with salts etc. Whey protein gels can be obtained by heating which proceeds through a series of transitions like denaturation of native proteins, aggregation of unfolded molecules, strand formation from aggregates or association of strands into a network. It should be noted that aggregates are formed in the presence of salts which results in the formation of strong gels. Likewise for soy proteins gelation process is obtained by heating soya bean flour or milk followed by addition of salt (e.g. Ca^{++}, or Mg^{++}) to from a gel (Cayot and Lorient 1997; Jong et al. 2009). In case of milk proteins casein molecules are strongly hydrophobic and thus micelles are hold together by hydrophobic bonds or salt bridges. Gels can be obtained by enzymatic hydrolysis of k-casein obtained from rennet CMP (caseinomacropeptide) and thus causes the micelles to aggregate resulting in rennet gelation. For egg proteins both albumen and yolk of liquid eggs have the capacity to form gels upon heating. Gel formation is a two-step process of denaturation followed by aggregation of denatured proteins (Montejano et al. 1984; Woodward and Cotterill 1986).

Emulsifying Properties

The amphiphillic nature of protein molecules is utilized to stabilize different types of emulsions. The stability of the emulsions by utilizing proteins comes from the fact that protein molecules concentrate at the oil and water interface; with lipophilic portion in the non-polar phase (oil) and the hydrophobic portion in the polar (water) phase (Wilde 2000). The stability is maximum when proteins form a solid viscoelastic structure which results in absorption, unfolding and formation of strong interactions. These interactions are well correlated with emulsion stability. The different method by which unfolding of proteins can be obtained are thermal, enzymatic, radiation and ultrasonic treatment. Some researchers have used combination of pH and heat treatment in order to change the native structure of protein molecule to impart desirable functional properties. Moreover, the unfolding of proteins at interfaces is influenced by the structure in solution, like flexible proteins will unfold quickly and rapidly and hence lower the interfacial tension (Kinsella and Whitehead 1989; Mitchell 1986), whereas globular proteins unfold more slowly as they have more intramolecular bonds stabilizing their structure (Wilde 2000). The unfolded proteins tend to form stronger intermolecular interactions and stabilize against coalescence very effectively (Mitchell 1986). Therefore, changing the structure of proteins by various means has been used as a tool for improving protein functionality, probably by inducing a change in adsorbed conformation. Emulsifying properties of proteins are important in many food systems. The important emulsifying properties of proteins include emulsion activity and emulsion stability. Emulsion activity is defined as the ability of a protein to form an emulsion by adsorbing oil at the oil-water-interface. On the other hand emulsion stability is the ability to stabilize emulsion without forming coalescence and flocculation over a period of time. These properties are important as they determine their ability to act as emulsifiers in various foods such as soup, sauce, confectionary product, and dairy products Karaca et al. (2011). These properties are greatly affected by molecular size, surface hydrophobicity, net charge, steric hindrance and molecular flexibility. Apart from this it has been observed that hydrophobic patches present on the surface of protein molecules are important for protein adsorption at the water oil interface during the formation of emulsion (Timilsena et al. 2016).

Film Forming Properties of Proteins

Biodegradable films developed from hydrocolloid materials are gaining tremendous interest due to their excellent mechanical, and comparable barrier properties. Proteins are well known for their film forming properties because they are far better than the films developed from polysaccharides and lipids. As protein molecules have unique structure due to the presence of 20 different monomers which are responsible for providing a wide range of functional properties, especially a high intermolecular binding potential. In addition to this the films formed from these hydrocolloid materials are usually biodegradable and safe packaging materials

thus reducing the pressures on landfill from plastic solid wastes. A large number of animal protein sources like milk proteins, collagen, gelatin, keratin, and myofibrillar protein are readily available for the development of biodegradable films. However due to rising global economic problems and consumer demands originated from health concerns, religious limitations and increasing trend of vegetarianism has recently arisen an interest in the usage of functional plant based proteins as alternative to animal proteins in the food industry for the development of biodegradable films (Dormont 2002; Alonso et al. 2006; Karim and Bhat 2009). Among plant sources the commonly used proteins sources are corn zein, wheat gluten, soy protein, amaranth protein, sunflower, chestnut proteins. Pseudo cereal proteins also are gaining popularity for the development of biodegradable films. The film forming ability is also associated with some desirable functional properties,such as barrier properties (i.e., water vapor permeability), mechanical properties (i.e., tensile strength, elongation, deformability, and elastic modulus) as well as microstructural properties (i.e., dough and fiber formation and texturizing capability) (Wihodo and Moraru 2013). These functional properties are crucial on improving the quality of food products, especially extending the shelf life of processed fruits and vegetables coated with the films. Some researchers have used polysaccharides in combination with proteins for the preparation of edible films however the applicability is limited due to their high water vapour permeability which is due to their hydrophilic nature. To improve the water-barrier properties of hydrocolloid-based films, lipid compounds are frequently incorporated into these structures causing a decrease in the WVP values at the expense of a reduction in the tensile strength and elasticity of the composite films (Morillon et al. 2002; Vargas et al. 2009). In addition to this the good film forming properties of plant proteins as compared to animal proteins makes them potential candidate materials for developing edible films which would serve as an alternative to plastic packaging materials (Bräuer et al. 2007).

In recent years bioactive films and coatings developed from proteins have received increasing attention. Nowadays, packaging plays a decisive role in the improvement of the shelf life of food products and new packaging materials derived from renewable sources are being developed (Lin and Zhao 2007). The potential of edible films to control gas transfer and to improve food quality, has received increasing attention from researchers and industry, possibly due to their numerous advantages over non-biodegradable plastic packaging films (Srinivasa et al. 2007). Edible film or coating can be defined as a thin, continuous layer of edible material formed or placed on or between foods or food components and poses no health hazard to consumers (Bravin et al. 2006). In addition to this edible films or coatings can also serve as a carrier of bioactive compounds, thus enhancing the functional properties of the food product by conferring number of health benefits. Most frequently used bioactive agents in edible films include lysozyme, oregano extract, chitosan, essential oils of clove, garlic and origanum, lactic acid (LA) and propionic acid (PRO), chitooligosaccharides and natamycin (NA) as antimicrobial agents. Incorporation of bioactive compounds to these films improves the functional properties such as water vapour permeability as well as antimicrobial and antioxidant properties

(Garcia et al. 2000; Oussalah et al. 2004; Seydim and Sarikus 2006).This can serve as a novel technique for packaging of many foods. Encapsulation can be a better approach for incorporation of bioactive compounds in edible films. Encapsulation of bioactive compounds into nano-vesicles may promote a number of beneficial effects by protecting them against degradation and undesirable interactions, and increasing their stability, apparent solubility and efficiency (Sozer and Kokini 2009; Brandelli et al. 2017). Besides, the amount of encapsulated bioactive required for a specific effect is often much less than the amount required when non-encapsulated (Reza Mozafari et al. 2008). Liposomes have been used as an interesting platform to deliver bioactive compounds, such as antimicrobials, antioxidants, vitamins in food systems (Fathi et al. 2012).

Protein Modification

The proteins have important functional properties from the technological point of view as they are amphiphillic in nature and the ability to from interfacial films also helps in stabilizing different food systems like emulsions and foam type foods. The stabilizing effect of food proteins is because of their large molecular weight which is associated with bulkier structure as compared with low molecular weight emulsifiers. After employing them in a particular food system they diffuse slowly to the oil water interface through the continuous phase. At the interface protein molecules undergoes surface denaturation and rearrange themselves in order to align their hydrophilic and hydrophobic groups in the oil and aqueous phase respectively thus ultimately results in the decrease in overall interfacial tension and free energy of the system (McClements 2004; Caetano da Silva Lannes and Natali Miquelim 2013).

However, native structure of protein is devoid of desirable functional traits and other important properties which are required in different food systems like creams, comminuted meat products, confectionary and dairy products. In order to increase the functionality of proteins several approaches are used which may be chemical, enzymatic and physical or a combination of these methods. As far protein functionality is concerned usually physical methods of protein modification are employed to achieve desirable functional properties as they are also safer than chemical methods. Moreover they are also considered as efficient methods in comparison with enzymatic approaches because enzymatic methods used for protein modification are usually time consuming. Some of the most commonly used physical methods for protein modification include radiation treatment (Electron beam, gamma and ultraviolet radiations), pulsed electric field, heat treatment and ultrasonic treatment. Modification of food proteins is usually carried out to alter the microstructure and physical performance of the biopolymers used for food, medical and industrial applications. Among the functional properties, the important ones which act as a target for modification include texture, flavor, color, solubility, foam stability, whippability and digestibility (Ball 1987; Hoogenkamp 2001). Table 2 lists different

Table 2 Different approaches used for increasing protein functionality

Approach	Type/modifying agent used	Reference
Physical	Pulsed electric field Heat treatment Ultrasonic treatment Elevated pressure treatment Electron beam irradiation Gamma irradiation Pulsed ultraviolet light Ozone processing	Fernandez-Diaz et al. (2000) Lam and Nickerson (2015) Resendiz-Vazquez et al. (2017) Guyon et al. (2018) Wang et al. (2017) Hassan et al. (2018) Meinlschmidt et al. (2016) Segat et al. (2014)
Chemical	Malondialdehyde modification Addition of succinyl groups PEGylation Acetylation Covalent modification by EGCG (−)-epigallocatechin-3-gallate Oxidative modification Hydrogen peroxide pH modification	Wang et al. (2018) Wan et al. (2018) He et al. (2018) Wang and Arntfield (2016) Jia et al. (2016) Duan et al. (2018) Sutariya and Patel (2017) Romani et al. (2018)
Enzymatic	Transglutaminase and thermolysin modification Corolase PP Alcalase Pepsin and trypsin treatment Corolase PP and flavourzyme hydrolysis Combined effect of alcalase, flavourzyme, neutrase, protamex, pepsin and trypsin	Damodaran and Li (2017) Guan et al. (2018) Ghribi et al. (2015) Ma et al. (2018) Connolly et al. (2014) Tang et al. (2009)

types of approaches which are responsible for increasing the functionality of proteins. It should be kept in mind that the type of technique chosen should not affect the other properties of proteins as they are usually sensitive to temperature and other processing conditions so well balance of processing parameters should be applied to obtain the desirable functional traits.

Conclusion and Future Directions

It is evident from the above discussion that proteins are the essential ingredients of our diet due to their tremendous applications, whether it may be nutritional or functional. The nutritional properties of proteins like essential amino acid index (EAAI), protein efficiency ratio (PER), biological value (BV) and amino acid score are the indicators of proteins overall quality so finding the best protein to be used in supplements and baby foods directly depends upon these properties. In addition to this, the structure of proteins also confers some important functional properties to various

food systems like bakery, creams, and comminuted meat products. Moreover there are some novel approaches by which proteins functional properties can be significantly improved for obtaining technological and functional attributes. Modifying approaches like ultrasound and radiation can be used as a green technology for imparting desirable traits. Polymeric materials like proteins can also be used for the encapsulation of antioxidants, minerals, fatty acids, probiotics and other bioactive compounds but these materials are associated with early and uncontrolled release due to their porous nature. Modification is a remedy for this problem as modification of food proteins is associated with the reduction in pore size which in turn increases the efficiency of these polymeric materials. Moreover correct dosage and desirable process parameters are to be taken into consideration which is key factor for increasing the overall efficacy of the process.

References

Alonso, A., Beunza, J. J., Bes-Rastrollo, M., Pajares, R. M., & Martínez-González, M. Á. (2006). Vegetable protein and fiber from cereal are inversely associated with the risk of hypertension in a Spanish cohort. *Archives of Medical Research, 37*(6), 778–786.

Ball, H. R., Jr. (1987). Functional properties of chemically modified egg white proteins. *Journal of the American Oil Chemists' Society, 64*(12), 1718–1725.

Brandelli, A., Lopes, N. A., & Boelter, J. F. (2017). Food applications of nanostructured antimicrobials. In *Food preservation* (pp. 35–74). London, UK: Elsevier.

Bräuer, S., Meister, F., Gottlöber, R.-P., & Nechwatal, A. (2007). Preparation and thermoplastic processing of modified plant proteins. *Macromolecular Materials and Engineering, 292*(2), 176–183.

Bravin, B., Peressini, D., & Sensidoni, A. (2006). Development and application of polysaccharide–lipid edible coating to extend shelf-life of dry bakery products. *Journal of Food Engineering, 76*(3), 280–290.

Caetano da Silva Lannes, S., & Natali Miquelim, J. (2013). Interfacial behavior of food proteins. *Current Nutrition & Food Science, 9*(1), 10e14. https://doi.org/10.2174/157340113804810914

Cayot, P., & Lorient, D. (1997). Surface-function relationships of whey proteins. In S. Damodaran & A. Paraf (Eds.), *Food proteins and their applications* (pp. 225–256). New York, NY: Marcel Dekker, Inc..

Connolly, A., Piggott, C. O., & FitzGerald, R. J. (2014). Technofunctional properties of a brewers' spent grain protein-enriched isolate and its associated enzymatic hydrolysates. *LWT - Food Science and Technology, 59*(2), 1061–1067.

Damodaran, S. (2008). Amino acids, peptides and proteins. In S. Damodaran, K. Parkin, & O. R. Fennema (Eds.), *Fennema's food chemistry*. Boca Raton, FL: CRC Press.

Damodaran, S., & Li, Y. (2017). A two-step enzymatic modification method to reduce immunoreactivity of milk proteins. *Food Chemistry, 237*, 724–732.

Dormont, D. (2002). Prion diseases: Pathogenesis and public health concerns. *FEBS Letters, 529*(1), 17–21.

Duan, X., Li, M., Shao, J., Chen, H., Xu, X., Jin, Z., & Liu, X. (2018). Effect of oxidative modification on structural and foaming properties of egg white protein. *Food Hydrocolloids, 75*, 223–228.

Fathi, M., Mozafari, M. R., & Mohebbi, M. (2012). Nanoencapsulation of food ingredients using lipid based delivery systems. *Trends in Food Science & Technology, 23*(1), 13–27.

Fernandez-Diaz, M. D., Barsotti, L., Dumay, E., & Cheftel, J. C. (2000). Effects of pulsed electric fields on ovalbumin solutions and dialyzed egg white. *Journal of Agricultural and Food Chemistry, 48*, 2332–2339.

Garcia, M. A., Martino, M. N., & Zaritzky, N. E. (2000). Lipid addition to improve barrier properties of edible starch-based films and coatings. *Journal of Food Science, 65*(6), 941–944.

Ghribi, A. M., Gafsi, I. M., Sila, A., Blecker, C., Danthine, S., Attia, H., … Besbes, S. (2015). Effects of enzymatic hydrolysis on conformational and functional properties of chickpea protein isolate. *Food Chemistry, 187*, 322–330.

Guan, H., Diao, X., Jiang, F., Han, J., & Kong, B. (2018). The enzymatic hydrolysis of soy protein isolate by Corolase PP under high hydrostatic pressure and its effect on bioactivity and characteristics of hydrolysates. *Food Chemistry, 245*, 89–96.

Guyon, C., Le Vessel, V., Meynier, A., & de Lamballerie, M. (2018). Modifications of protein-related compounds of beef minced meat treated by high pressure. *Meat Science, 142*, 32.

Hassan, A. B., Mahmoud, N. S., Elmamoun, K., Adiamo, O. Q., & Ahmed, I. A. M. (2018). Effects of gamma irradiation on the protein characteristics and functional properties of sesame (Sesamum indicum L.) seeds. *Radiation Physics and Chemistry, 144*, 85–91.

He, Q., Sun, X., He, S., Wang, T., Zhao, J., Yang, L., & Sun, H. (2018). PEGylation of black kidney bean (Phaseolus vulgaris L.) protein isolate with potential functironal properties. *Colloids and Surfaces B: Biointerfaces, 164*, 89–97.

Hoogenkamp, H. W. (2001). *Soy protein and meat formulations*. Oxford: CABI Publishing.

Jia, Z., Zheng, M., Tao, F., Chen, W., Huang, G., & Jiang, J. (2016). Effect of covalent modification by (−)-epigallocatechin-3-gallate on physicochemical and functional properties of whey protein isolate. *LWT - Food Science and Technology, 66*, 305–310.

Jong, S. H., Klok, J., & Velde, F. V. (2009). The mechanism behind microstructure formation in mixed whey protein–polysaccharide cold-set gels. *Food Hydrocolloids, 23*, 755–764.

Karaca, A. C., Low, N., & Nickerson, M. (2011). Emulsifying properties of canola and flaxseed protein isolates produced by isoelectric precipitation and salt extraction. *Food Research International, 44*(9), 2991–2998.

Karim, A. A., & Bhat, R. (2009). Fish gelatin: Properties, challenges, and prospects as an alternative to mammalian gelatins. *Food Hydrocolloids, 23*(3), 563–576.

Kinsella, J. E., & Whitehead, D. M. (1989). Proteins in whey: Chemical, physical, and functional properties. In J. E. Kinsella (Ed.), *Advances in food and nutrition research* (Vol. 33, pp. 343–438). San Diego, CA: Academic Press.

Lam, R. S., & Nickerson, M. T. (2015). The effect of pH and temperature pre-treatments on the physicochemical and emulsifying properties of whey protein isolate. *LWT - Food Science and Technology, 60*(1), 427–434.

Li-Chan, E. C. Y. (2012). Proteins: Basic concepts. In Y. H. Hui (Ed.), *Food chemistry: Principles and applications*. West Sacramento, CA: Science Technology Systems.

Lin, D., & Zhao, Y. (2007). Innovations in the development and application of edible coatings for fresh and minimally processed fruits and vegetables. *Comprehensive Reviews in Food Science and Food Safety, 6*(3), 60–75.

Ma, S., Wang, C., & Guo, M. (2018). Changes in structure and antioxidant activity of β-lactoglobulin by ultrasound and enzymatic treatment. *Ultrasonics Sonochemistry, 43*, 227–236.

McClements, D. J. (2004). Protein-stabilized emulsions. *Current Opinion in Colloid & Interface Science, 9*(5), 305–313. https://doi.org/10.1016/j.cocis.2004.09.003

Meinlschmidt, P., Ueberham, E., Lehmann, J., Reineke, K., Schlüter, O., Schweiggert-Weisz, U., & Eisner, P. (2016). The effects of pulsed ultraviolet light, cold atmospheric pressure plasma, and gamma-irradiation on the immunoreactivity of soy protein isolate. *Innovative Food Science & Emerging Technologies, 38*, 374–383.

Mir, N. A., Riar, C. S., & Singh, S. (2018). Nutritional constituents of pseudo cereals and their potential use in food systems: A review. *Trends in Food Science & Technology, 75*, 170–180.

Mitchell, J. R. (1986). Foaming and emulsifying properties of proteins. In B. J. Hudson (Ed.), *Developments in food proteins* (Vol. 4, pp. 291–338). London, UK: Elsevier.

Montejano, J. G., Hamann, D. D., Ball, H. R., Jr., & Lanier, T. C. (1984). Thermally induced gelation of native and modified egg white-rheological changes during processing; final strengths and microstructures. *Journal of Food Science, 49*, 1249–1257.

Morillon, V., Debeaufort, F., Blond, G., Capelle, M., & Voilley, A. (2002). Factors affecting the moisture permeability of lipid-based edible films: A review. *Critical Reviews in Food Science and Nutrition, 42*(1), 67–89.

Nelson, D. L., & Cox, M. M. (2013). *Lehninger principles of biochemistry*. New York, NY: Worth Publishers.

Osborne, T. B. (1924). *The vegetable proteins* (p. 154). London, UK: Longmans.

Oussalah, M., Caillet, S., Salmiéri, S., Saucier, L., & Lacroix, M. (2004). Antimicrobial and antioxidant effects of milk protein-based film containing essential oils for the preservation of whole beef muscle. *Journal of Agricultural and Food Chemistry, 52*(18), 5598–5605.

Resendiz-Vazquez, J. A., Ulloa, J. A., Urías-Silvas, J. E., Bautista-Rosales, P. U., Ramírez-Ramírez, J. C., Rosas-Ulloa, P., & González-Torres, L. (2017). Effect of high-intensity ultrasound on the technofunctional properties and structure of jackfruit (Artocarpus heterophyllus) seed protein isolate. *Ultrasonics Sonochemistry, 37*, 436–444.

Reza Mozafari, M., Johnson, C., Hatziantoniou, S., & Demetzos, C. (2008). Nanoliposomes and their applications in food nanotechnology. *Journal of Liposome Research, 18*(4), 309–327.

Romani, V. P., Machado, A. V., Olsen, B. D., & Martins, V. G. (2018). Effects of pH modification in proteins from fish (Whitemouth croaker) and their application in food packaging films. *Food Hydrocolloids, 74*, 307–314.

Salunkhe, D. K., Chavan, J. K., Adsule, R. N., & Kadam, S. S. (1992). Sunflower. In *World oilseeds: Chemistry, technology and utilization* (pp. 97–139). New York, NY: Van Nostrand Reinhold Publishing.

Segat, A., Misra, N. N., Fabbro, A., Buchini, F., Lippe, G., Cullen, P. J., & Innocente, N. (2014). Effects of ozone processing on chemical, structural and functional properties of whey protein isolate. *Food Research International, 66*, 365–372.

Seydim, A. C., & Sarikus, G. (2006). Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. *Food Research International, 39*(5), 639–644.

Shewry, P. R., Halford, N. G., Belton, P. S., & Tatham, A. S. (2002). The structure and properties of gluten: An elastic protein from wheat grain. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci., 357*, 133–142.

Sozer, N., & Kokini, J. L. (2009). Nanotechnology and its applications in the food sector. *Trends in Biotechnology, 27*(2), 82–89.

Srinivasa, P. C., Ramesh, M. N., & Tharanathan, R. N. (2007). Effect of plasticizers and fatty acids on mechanical and permeability characteristics of chitosan films. *Food Hydrocolloids, 21*(7), 1113–1122.

Stefanović, A. B., Jovanović, J. R., Dojčinović, M. B., Lević, S. M., Nedović, V. A., Bugarski, B. M., & Knežević-Jugović, Z. D. (2017). Effect of the controlled high-intensity ultrasound on improving functionality and structural changes of egg white proteins. *Food and Bioprocess Technology, 10*(7), 1.

Sutariya, S., & Patel, H. (2017). Effect of hydrogen peroxide on improving the heat stability of whey protein isolate solutions. *Food Chemistry, 223*, 114–120.

Tandang-Silvas, M. R., Cabanos, C. S., Carrazco Peña, L. D., De La Rosa, A. P. B., Osuna-Castro, J. A., Utsumi, S., … Maruyama, N. (2012). Crystal structure of a major seed storage protein, 11S proglobulin, from Amaranthus hypochondriacus: Insight into its physico-chemical properties. *Food Chemistry, 135*, 819–826.

Tang, C. H., Wang, X. S., & Yang, X. Q. (2009). Enzymatic hydrolysis of hemp (Cannabis sativa L.) protein isolate by various proteases and antioxidant properties of the resulting hydrolysates. *Food Chemistry, 114*(4), 1484–1490.

Taylor, J. R. N., Taylor, J., Campanella, O. H., & Hamaker, B. R. (2016). Functionality of the storage proteins in gluten-free cereals and pseudocereals in dough systems. *Journal of Cereal Science, 67*, 22–34.

Timilsena, Y. P., Adhikari, R., Barrow, C. J., & Adhikari, B. (2016). Physicochemical and functional properties of protein isolate produced from Australian chia seeds. *Food Chemistry, 212*, 648–656.

Vargas, M., Albors, A., Chiralt, A., & González-Martínez, C. (2009). Characterization of chitosan–oleic acid composite films. *Food Hydrocolloids, 23*(2), 536–547.

Voet, D., Voet, J. G., & Pratt, C. W. (2013). *Fundamentals of biochemistry, life at the molecular level* (4th ed.). Hoboken, NJ: John Wiley & Sons.

Wan, Y., Liu, J., & Guo, S. (2018). Effects of succinylation on the structure and thermal aggregation of soy protein isolate. *Food Chemistry, 245*, 542–550.

Wang, J., Zhao, M., Qiu, C., & Sun, W. (2018). Effect of malondialdehyde modification on the binding of aroma compounds to soy protein isolates. *Food Research International, 105*, 150–158.

Wang, K., & Arntfield, S. D. (2016). Modification of interactions between selected volatile flavour compounds and salt-extracted pea protein isolates using chemical and enzymatic approaches. *Food Hydrocolloids, 61*, 567–577.

Wang, L., Ding, Y., Zhang, X., Li, Y., Wang, R., Luo, X., & Chen, Z. (2017). Effect of electron beam on the functional properties and structure of defatted wheat germ proteins. *Journal of Food Engineering, 202*, 9–17.

Wihodo, M., & Moraru, C. I. (2013). Physical and chemical methods used to enhance the structure and mechanical properties of protein films: A review. *Journal of Food Engineering, 114*(3), 292–302.

Wilde, P. J. (2000). Interfaces: Their role in foam and emulsion behaviour. *Current Opinion in Colloid & Interface Science, 5*, 176–181.

Woodward, S. A., & Cotterill, O. J. (1986). Texture and microstructure of heat-formed egg white gels. *Journal of Food Science, 51*, 333–339.

Zhu, H., & Damodaran, S. (1994). Heat-induced conformational changes in whey protein isolate and its relation to foaming properties. *Journal of Agricultural and Food Chemistry, 42*(4), 846–855.