Chapter 3

Protein-Based Edible Films: Characteristics and Improvement of Properties

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1. Introduction

Protein-based edible films have received consideration attention in recent years because of their advantages, including their use as edible packaging materials, over the synthetic films. In addition, protein-based edible films can also be used for the individual packaging of small portions of food, particularly products that are not currently individually packaged for practical reasons, such as beans, nuts and cashew nuts. In addition, protein-based edible films can be applied inside heterogeneous foods at the interfaces between different layers of components. They can be tailored to prevent the deterioration of inter-component moisture and solute migration in foods such as pizzas, pies and candies. Moreover, protein-based edible films can function as carriers for antimicrobial and antioxidant agents. Through a similar application they also can be used at the surface of food to control the diffusion rate of preservative substances from the surface to the interior of the food. Another possible application for protein-based edible films could be their use in multilayer food packaging materials together with non edible films. In this case, the protein-based edible films would be the internal layers in direct contact with food materials. Through functions relating to mechanical and barrier properties, protein-based edible films may be able to substitute synthetic polymer films.

In their natural states, proteins generally exist as either fibrous proteins or globular proteins. The first type are water insoluble and serve as the main structural materials of animal tissues., Globular proteins are soluble in water or aqueous solutions of acids bases or salts and function widely in living systems. The fibrous proteins are fully extended and associated closely with each other in parallel structures, generally through hydrogen bonding, to form fibers. The globular proteins fold into complicated spherical structures held together by a combination of hydrogen, ionic, hydrophobic and covalent (disulfide) bonds. The chemical and physical properties of these proteins depend on the relative amounts of component amino acid residues and their placement along the protein polymer chain. Regarding the fibrous proteins, collagen has received the most attention in the production of edible films. Several globular proteins, including wheat gluten, corn zein, soy protein, whey protein and mung bean protein, have been investigated for their film properties.

Protein-based edible films are generally formed from solutions or dispersions of the protein as the solvent/carrier evaporates. The solvent/carrier is generally limited to water, ethanol or ethanol-water mixtures. Generally, proteins must be denatured by heat, acid, bases, and/or solvents in order to form the more extended structures that are required for film formation. Once extended, protein chains can associate through hydrogen, ionic, hydrophobic and covalent bonding. The chain-to-chain interaction that produces cohesive films is affected by the degree of chain extension and the nature and sequence of amino acid residues. The uniform distribution of polar, hydrophobic, and/or thiol groups along the polymer chain increase the likelihood of the respective interactions. The promotion of polymer chain-to-chain interaction results in films that are stronger but less flexible and less permeable to gases, vapors and liquids. Polymers containing groups that can associate through hydrogen or ionic bonding result in films that are excellent oxygen barriers but susceptible to moisture. Thus, protein films are expected to be good oxygen barriers at low relative humidity.

Polymers containing a preponderance of hydrophobic groups are poor oxygen barriers but excellent moisture barriers. However, the fact that proteins are not totally hydrophobic and contain predominantly hydrophillic amino acid residues limits their moisture-barrier properties. The creation of protein to edible films with low water vapor permeability requires the addition of hydrophobic components. This is analogous to the situation with synthetic polymers. Here moisture-sensitive oxygen-barrier polymers must be either co polymerized with a hydrophobic polymer or sandwiched between hydrophobic polymer layers to limit the ability of water to reduce barrier properties. Because of the poor water vapor resistance of protein-based edible films and their lower mechanical strength in comparison with synthetic polymers their application is limited in food packaging. The improvement of protein-based edible films properties has been investigated to seek suitable applications. This chapter provides details of protein-based edible films and their properties, the production of protein-based edible films, the methods used for the formation of protein-based edible films, the improvement of properties of protein-based edible films and their potential applications.

2. Type of protein-based edible films

2.1. Collagen films

Collagens are the major structural proteins of connective tissue such as bone, hide, tendons cartilage, and ligaments. They constitute about one-third of the total body protein in mammals. There are different types of collagen. Each type has its own amino acid sequence, but all contain a significant amount of triple helical structure. Because of its biological properties and ready availability, type I collagen is widely used as a biomaterial. It is used in a variety of physical forms such as sponges, films and membranes (Sisken et al., 1993). Collagen possesses characteristics as a biomaterial that is distinct from those of synthetic polymers. Collagen is used to make the most commercially successful edible protein films. Collagen films offer several advantages: it is biocompatible and non-toxic to most tissues; it has well-documented structural, physical, chemical, and immunological properties; it can be processed into a variety of forms; and it is readily isolated and purified in large quantities. The production of collagen films from animal hides can be accomplished using a dry or wet process with some similarities. These include: (a) alkaline treatment to de-hair and remove collagen from carbohydrates and other proteins; (b) acid swelling and homogenization to form a ~ 4.5% moisture gel (wet process) or ~ 10% moisture gel dough (dry process); (c) extrusion into a tube; and (d) neutralization of the extruded tube, washing the tube of salts, treating the tube with plasticizer and cross-linkers and drying to 12-14% moisture (the order depends on whether the wet or dry process is used) (Hood, 1988).

2.2. Gelatin films

Gelatin is unique among hydrocolloids in forming a thermo-reversible substance with a melting point close to body temperature, which is particularly significant in edible and pharmaceutical applications. Basically, gelatin is obtained by controlled hydrolysis from the fibrous insoluble protein, collagen, which is widely found in nature as the major constituent of skin, bones and connective tissue. Gelatin is composed of a unique sequence of amino acids. The characteristic features of gelatin are the high content of the amino acids glycine, proline and hydroxyproline. Gelatin also has a mixture of single and double unfolded chains of a hydrophilic character (Ross-Murphy, 1992). At approximately 40 oC, gelatin aqueous solutions are in the sol state and form physical, thermoreversible gels on cooling. During gelation, the chains undergo a conformational disorder–order transition and tend to recover the collagen triple-helix structure (Ross-Murphy, 1992).

Gelatin is used to encapsulate low moisture or oil phase food ingredients and pharmaceuticals. Such encapsulation provides protection against oxygen and light, as well as defining the amount of ingredient or drug dosage. In addition, gelatin films have been formed as coatings on meats to reduce oxygen, moisture and transport of oil (Gennadios et al., 1994). Addition, gelatin is able to form clear and strong films and is used for microencapsulation and capsule coatings in food and pharmaceutical manufacturing. Gelatin films could be formed from 20-30% gelatin, 10-30% plasticizer (glycerin or sorbitol) and 40-70% water followed by drying the gelatin gel (Guilbert, 1986). However, gelatin films, as with most protein films, do not have an ideal water vapor barrier, which limits its application as edible film and biomaterial. However, modification of the polymer network through cross-linking of the polymer chains can be applied to improve the functionality of protein film.

2.3. Corn zein films

Zein is the most important protein in corn. It is a prolamin protein and therefore dissolves in 70–80% ethanol (Dickey & Parris, 2002). Zein is a relatively hydrophobic and thermoplastic material. The hydrophobic nature of zein is related to its high content of non-polar amino acids (Shukla & Cheryan, 2001). Technically, the films made from an alcohol soluble protein like zein, have relatively high barrier properties compared to other proteins. Zein has excellent film forming properties and can be used for the fabrication of biodegradable films. Zein film is formed through the development of hydrophobic, hydrogen and limited disulfide bonds between zein chains (Guilbert, 1986). The formation of corn zein films is believed to involve the development of hydrophobic, hydrogen and limited disulfide bonds between zein chains in the film matrix (Gennadios et al., 1994). The resulting films are brittle and therefore require the addition of plasticizer for increasing the flexibility. Zein films are relatively good water vapor barriers compared to other edible films (Guilbert, 1986). Zein coating have also shown an ability to reduce moisture and loss of firmness and delay color change (the reduction of oxygen and carbon dioxide transmission) in fresh fruit. In addition, zein may also take part in the coating of conventional packaging plastics. Although, zein is definitely not water soluble at a neutral pH, it has high water vapor permeability compared with typical synthetic polymers. However, the water vapor barrier properties can be improved by adding fatty acids or by using a cross-linking reagent. However, when cross-linking agents are used, the edibility of the resulting films needed to be considered.

2.4. Wheat gluten films

Wheat gluten is a water insoluble protein of wheat flour which is comprised of a mixture of polypeptide molecules, and considered to be globular proteins. The cohesiveness and elasticity of the gluten gives integrity to wheat dough and facilitates film formation. Wheat gluten is composed of two main groups of water insoluble proteins: gliadins, consisting of low molecular weight proteins; and glutenins containing high molecular weight proteins. Gliadins are single monomeric proteins in which disulfide bonds make up intra-chains or are absent, while glutenins form high molecular weight polymers maintained by inter-chain disulfide bonds. Films from glutenins were stronger and had better barrier properties than films from gliadins or whole gluten. Gliadin films presented better optical properties but were not water resistant. The properties of these films make them sometimes sensitive to thermal treatments, which could result in improved film properties. Addition, gliadin is soluble in 70% ethanol; glutenin is not (Gennadios & Weller, 1990). Although insoluble in natural water, wheat gluten dissolves in aqueous solutions of high or low pH at low ionic strength (Krull & Inglett, 1971). Wheat gluten films can be fully biodegraded after 36 days in aerobic fermentation and within 50 days in farmland soil without releasing toxic products (Domenek et al., 2004).

Technically, wheat gluten films can be formed by drying aqueous ethanol solutions. Cleavage of native disulfide bonds during the heating of film-forming solutions. The formation of new disulfide bonds during film drying is then believed to be important to the formation of wheat gluten films structure, along with hydrogen and hydrophobic bonds (Gennadios & Weller, 1990). The addition of plasticizer such as glycerin in gluten films is necessary to improve the flexibility of wheat gluten films. However, increasing film flexibility by increasing plasticizer content may reduce the strength, elasticity and water vapor barrier properties of the resulting film. In addition, the purity of wheat gluten also affects the film's appearance and mechanical properties; greater purity gluten results in stronger and clearer films. Wheat gluten films are effective oxygen barriers, but poor water vapor barriers. The poor resistance of wheat gluten films to water vapor is due to the hydrophilic nature of the protein and to the substantial amount of hydrophilic plasticizer added to impart adequate film flexibility. The properties of wheat gluten films can be improved by using a cross-linking agent such as glutaraldehyde, or heat curing.

2.5. Soy protein films

Soy protein from soybeans has been extensively used as a food ingredient in nearly every food product available to the consumer, since it contains high nutrition and excellent functional properties. The protein content of soybeans (38-44%) is much higher than the protein content of cereal grain (8-15%). The major soybean proteins have molecular weights ranging from 200 to 600 kDa. Most soy proteins (90%) are globulins, which can be fractionated into 2S, 7S, 11S and 15S according to their sedimentation coefficients. 7S and 11S are the main fractions, being about 37% and 31% of the total extractable protein and have a capacity for polymerization (Cho & Rhee, 2004). Soy proteins consist of both polar and non-polar side chains. There are strong intra- and inter-molecular interactions, such as hydrogen bonding, dipole–dipole, charge–charge, and hydrophobic interactions. The strong charge and polar interactions between side chains of soy protein molecules restricts segment rotation and molecular mobility, which increase the stiffness, yield point, and tensile strength of soy protein films (Zhang et al., 2001).

Because soy proteins are abundant, inexpensive, biodegradable, and nutritional, they show the potential to be developed as edible and biodegradable films. The formation of films from soy proteins has been described as a two-step process involving: (a) the heating of film solutions to disrupt the protein structure, cleave native disulfide bonds and expose sulfhydryl groups and hydrophobic groups; and (b) the formation of new disulfide, hydrophobic and hydrogen bonds. The unfolded proteins link through intermolecular interactions, such as the disulfide bonds and hydrophobic interactions, leading to the formation of a network which occur during drying processes.

Technically, protein-based edible films can form bonds at different positions and offer high potential for forming several linkages. However, soy protein films still have poor moisture barrier properties due to their hydrophilic properties and the substantial amount of hydrophilic plasticizer used in film preparation. One extensively used method to enhance the water vapor barrier of films has been the incorporation of hydrophobic compounds such as lipids into the film forming solution. In addition, the other way to improve the properties of soy protein film is to modify the protein network through cross-linking of the protein chains. The presence of reactive functional groups in the amino acid side chain of protein makes this cross-linking process possible through chemical, enzymatic or physical treatments.

2.6. Casein films

Milk proteins can be classified into two types: casein and whey protein. Casein comprises of three principal components,α, ß, and κ, which together form colloidal micelles in milk containing large numbers of casein molecules and are stabilized by a calcium-phosphate bridge (Kinsella, 1984). The casein molecules possess little defined secondary structure, exhibiting instead an open random-coil structure. Casein, which comprises 80% of milk protein, precipitates when skim milk is acidified to the casein isoelectric point of approximately 4.6 (Dalgleish, 1989). Acidification solubilizes the calcium phosphate, thus releasing individual casein molecules, which associate to form insoluble acid casein. The acid casein can be converted to functional soluble caseinates by neutralization through the addition of alkali. Edible protein films based on various caseinates can be obtained by solubilization in water followed by casting and drying.

Caseinates films are made from aqueous solutions without heat treatment due to their random coil nature. Interactions in the film matrix are likely to include hydrophobic, ionic, and hydrogen bonding (Avena-Bustillos & Krochta, 1993). Caseinate films are transparent and flexible, but have poor water barrier properties. At comparable test conditions, caseinate films appear to have similar moisture barriers to wheat gluten films and soy protein films but poorer moisture barriers than corn zein films. Casein has been investigated for the formation of free standing films and coatings on food products. Laminated films that included casein did protect dried fruit and vegetables from moisture absorption and oxidation. Caseinate-lipid emulsion coatings were successful in reducing moisture loss from peeled carrots and zucchini (Avena-Bustillos et al., 1993).

2.7. Mung bean protein films

Mung beans are of interest as a potential component of biopolymeric films because of their high protein content. The whole seeds of mung beans contain approximately 25-30% protein. The proteins from mung bean are large and much of the protein has a molecular weight (MW) between 24 and 55 kDa with some traces having less than 24 kDa. However, there were small amounts of proteins of a MW between 24 and 14.2 kDa. The amino acid composition of mung bean means they are rich in essential amino acids such as leucine, isoleucine, lysine, and phenylalanine and are also rich in acidic amino acids such as glutamic acid and aspartic acid. However, the sulfur containing amino acid, such as methionine and cysteine, were also detected in mung bean protein (2.75 and 3.62%) (Keereekasetsuk et al., 2009).

Bourtoom (2008) prepared and analyzed the films from mung bean protein. It was found that the mechanical properties (tensile strength and elongation at break) of mung bean protein films had superior mechanical properties and water vapor barrier properties. These were better than other protein sources such as casein, soy protein isolate, wheat gluten, peanut proteins and water-soluble fish proteins films. However, the mung bean protein films still showed substantially lower mechanical and water vapor barrier properties compared with some synthetic polymers (high density polyethylene, polyvinyl chloride, cellulose acetate and polyester). The resistance of protein mung bean protein films to water vapor permeability is limited due to the inherent hydrophilicity of proteins. The transmission of water vapor through protein-based edible film is also facilitated by the presence of, a hydrophilic plasticizer, which favors adsorption of water molecules. However, the properties of mung bean protein films can be improved by addition of hydrophobic materials and also using chemical and enzymatic cross linking.

3. Formation of protein-based edible films

Protein-based edible films may be formed by two different methods: surface film formation and the deposition method.

3.1. Surface films formation

Films are obtained by the prolonged heating of film solutions and films are periodically harvested from the surface, drained and dried. The use of this method has been described as a two-step process involving the heat denaturation of the proteins followed by surface dehydration. Heating changes the three-dimensional structure of proteins and the existing functional groups. These, such as CO and NH of peptidic bonds, side chain amine groups and hydrophobic groups, engage in intra-molecular hydrogen bonding and electrostatic interaction (Wang & Damodaran, 1991). During drying, the unfolded proteins approach each other and become linked through intermolecular interactions (disulfide and hydrophobic interaction). This yields the formation of a protein network that acts as the matrix for entrapping film components such as plasticizing agents (Gennadios & Weller, 991). When the formation of film occurs in denaturated conditions, it is assumed that the protein remains in the fully denaturated state in the film.

However, it is quite possible that the denatured protein may undergo partial refolding, thus regaining some secondary structure during the film process. It is conceivable that the extent of such refolding affects the number of functional groups available for intermolecular interactions and thus the formation and stability of the film network (Subirade et al, 1998). Wu & Bates (1973) have prepared films from peanut milk. They reported that during the heating of the peanut milk, the high molecular weight peanut protein is broken down into lower molecular weight moiety. Heat first dissociates the conarachin and then arachin fractions to form small subunits of insoluble complexes at the surface. In addition, the interfacial forces may initiate the formation of protein matrixes capable of trapping oil droplets and water released from the surface facilitating the formation of protein matrixes (Farnum et al., 1976).

3.2. Deposition method

Films obtained from this method generally are made by casting and drying film forming solution on a non-stick surface. Technically, the casting process consists of drying a film solution or a gel for producing films with controlled thickness. This technique is useful to mimic some industrial processes for forming free standing starch films as is the case for dip-molding. In this method, which is used for food coatings as well as for non-food applications, the gelled state is usually preferred to set hot solutions on a surface upon cooling. Jaynes & Chou (1975) used this method to produce soy protein-lipid films. They used a protein isolate solution at natural pH 6.6 casting on Teflon coated baking pan and drying at 100 °C.

Films made from deposition techniques are more uniform films compared to the surface forming method. The film thickness can be controlled by the amount of total solid in the film solutions which is not the case when the surface formation method is employed. Most researchers have been using the deposition technique in recent years to produce edible films. However, casting material and casting temperature may vary depending upon the state and type of substrate. The deposition technique has been used to make protein films from wheat gluten, corn zein, casein, whey protein isolate, soy protein isolate and rice protein concentrate.

4. Factors affecting protein-based edible films

4.1. Type of raw material

Raw materials used in film solutions are classified, according to their solubility characteristics, into two categories-hydrophilic and hydrophobic. Hydrophilic materials such as soy protein isolate, whey protein isolate, water soluble fish protein and mung bean proteins are water soluble. Hydrophobic materials such as corn zein, wax are water-insoluble but they dissolve in non-polar liquids such as alcohol. The difference in soluble properties of these raw materials influence the amount of energy needed to obtain dried films and their use on foods. Carbohydrates such as alginate, carregeenan, pectin, starch, cellulose and cellulose derivatives provide a strong matrix free standing film, but these films are poor water barrier properties because of the hydrophilic nature of raw materials used (Kester and Fennema, 1986). Proteins provide good gas barrier but poor water vapor barrier properties. However, some protein films such as corn zein films exhibit better water resistance than other protein films because zein contains high amount of hydrophobic side chain amino acid. Lipid films, made from hydrophobic materials such as wax, fatty acid, show excellent water vapor barriers but poor mechanical properties.

4.2. Polymer chemistry

The regular structure molecule is more diffusible than the irregular stereochemical structure whereas branched molecules may provide a greater cohesive strength than non-branched molecules. A lower molecular weight fraction shows a greater cohesion and a greater change in cohesion with temperature change. In highly polar polymers such as protein and cellulosic, self-adhesion by diffusion is not significant due to the minimal flexibility and fixed order of the macromolecule. This is caused by the internal molecular forces holding the polymer chains. Cellulosics have a back bone with a rigid ring structure chain whereas proteins tend to form helical chain structure (Banker, 1966).

Kinsellar & Phillips (1989) summarized the desired molecular characteristic for formation of protein films: 1) high soluble molecules promote rapid diffusion; 2) the large molecules allow more interactions at the interface resulting in strong film; 3) amphiphatic molecules provide an unbalanced distribution of charged and apolar residuals for improved interfacial interaction; 4) flexible domains facilitate phase behavior and unfolding at interface; 5) the dispersion of charged groups affect protein-protein interaction in the films and charge repulsion between neighboring bubbles; 6) polar residue can provide hydratable or charged residues to keep bubbles apart, and binding and retaining water; 7) the retention of structure could be enhance overlap and segmental interaction in film; and 8) interactive regions can affect the deposition of different functional segments and facilitate secondary interactions in the air and aqueous phases.

4.3. pH

pH plays an important role in protein films made from water-soluble materials, such as soy protein isolate and whey protein isolate, as the solubility of these proteins depend on their isoelectric point (pI). During the dissolution of macromolecular substances, the cohesive forces between the solute macromolecules are neutralized by unions with the solvent molecules (Banker, 1966). The functionality of the polymer is related to the solution's properties which further influences film characteristics. The charge groups repel each other and produce a stretching of the polymer chain when the functional groups on a linear polymer become ionized during dissolution. The greater the degree of dissolution and the more extensively the chain is charged, the greater is the uncoiling of the chain.

The interaction between the charged polymer molecules and the molecules of the polar solvent increases with the increasing charge on the chain. The maximum protein solubility is obtained at pH away from its isoelectric point (pI). But to produce an edible film at extreme pH, the sensory property must also be considered along with other film properties. Gennadios et al. (1993) studied the effect of pH on soy protein isolate film and found that highly acidic ($pH < 1$) or alkaline conditions ($pH > 12$) inhibit the formation of soy protein isolate film. Kinsella & Phillip (1989) reported that films formed near the isoelectric point of major proteins are more condensed and stronger.

4.4. Drying temperature

Protein-based edible films are usually obtained by the casting method. This technique involves the drying of a complex colloidal solution made up of the protein, a solvent and, usually, a plasticizer previously poured on an appropriate support. The effect of a specific drying temperature depends on the various characteristics of the raw material, such as the occurrence of a preexisting gel phase or the occurrence of thermal gelation during drying. Furthermore, various phenomena may occur such as the transition from a rubbery to a vitreous phase, a phase separation (thermodynamic incompatibility) or crystallization. The interaction between the physicochemical nature of biopolymers and the drying conditions is very important (Devani et al., 2009).

The interaction forces in protein structures are affected by temperature. The temperature is a strong denaturing factor for proteins, and even the thermal stability and conformation of protein depend on the amino acid composition. During the drying period, when water is progressively eliminated, the conformation of the proteins change. Furthermore, the degree of protein unfolding determines the type and proportion of covalent (S–S bonds) or non-covalent (hydrophobic interactions, ionic and hydrogen bonds) interactions that can be established between protein chains. It is known that chains can interact more strongly and easily, especially through disulfide bonds, when proteins are denatured (Mauri & Anon, 2006). The cohesion of the final network would then be a function of these bonds and determines the properties of the films obtained.

In addition, hydrophillic interactions increase, hydrogen bonds and electrostatic interaction decreases when temperature increases. This results in the facilitation of adhesion between polymer films and the substrate (Banker, 1966). High temperature (70-100 ^{o}C) affects the forming of rigid structures in protein solutions because of protein denaturation (Chefel et al., 1986). Excessive heat or an excessive solvent evaporation rate during processes may produce non-cohesive films (Guilbert et al., 1986). Water soluble proteins such as soy protein and whey protein need a higher temperature and longer time for film formation than films from alcohol-soluble protein such as corn zein or wheat gluten. The higher drying temperature of water-soluble based-films may limit a film's use. However, low relative humidity can also be employed for film formation at low temperature

4.5. Concentration

Protein films make up complex structures. Protein–protein interactions within aggregates can be of a different nature (such as electrostatic or hydrophobic, etc.), which can lead to different kinds of cohesiveness. This could affect the mobility of proteins and their ability to form films. The concentration of film solutions affect the self adhesion of high polymers and the rate of matrix forming in film preparations. Besides, the protein concentration in film solution can also influence the formation of the protein matrix. At a lower protein concentration there is probably less protein-protein interaction, while at the higher protein concentration self-diffusion is promoted resulting in inferior properties. At the optimum concentration of film solutions, an intermediate viscosity could be obtained which result in the highest cohesive strength. However, the optimum concentration of each protein films requires various concentrations. The production of films with whey protein isolate requires a relatively high protein concentration (>8%) in the film forming solution so that the formation of S–S bridges occur (Sothornvit & Krochta, 2001). Whereas the films produced from muscle fish protein prepared with 1.5-2% showed stronger films than other concentrations.

4.6. Relative humidity

Water interaction with protein-based edible films are considered a priority in order to explain the physical property modifications induced by the presence of the moisture content. The adsorption of water vapor by dried materials is generally assumed to involve the binding of water molecules to specific hydrophilic sites, such as carboxylic, amino and hydroxy residues, in addition to backbone peptide groups. At high relative humidity, multimolecular adsorption occurs through swelling and conformational changes in the macromolecular structure. Basically, the property of protein-based edible films varies to the same degree as storage time, especially if compared with those of synthetic films, due to the intrinsic instability of their raw materials. These variations could affect their properties as a result of changing characteristics. Thus the oxidation of the protein sulfhydryl groups could cause degradation of the polymeric chains (Micard et al., 2000). Physical changes include polymer rearrangement and may be due to the migration of the low molecular weight components used in film formulation, such as plasticizers (Anker et al., 2001). The migration of additives could be considered the most important cause of physical instability of protein films.

The relationship between equilibrium relative humidity and film water content has been assessed by measuring water sorption isotherms. In addition, an understanding of water sorption properties is necessary in order to tailor film applications. The influence of relative humidity on the mechanical properties and permeability of protein film has been tentatively explained by the decrease of glass transition temperature induced by a plastification phenomena created by the water on protein films.

Cuq et al. (1996) determined the effect of relative humidity on the mechanical and water vapor barrier properties of myofibrillar protein-based films. The plasticizing effect of water related to rapid changes in the functional properties of the resulting films was explained by disruptive water-polymer hydrogen bonding. Relatively sharp decreases in force at break, the elastic modulus and water vapor barrier properties, and increases in deformation at break were observed when relative humidity increased. Pochat-Bohatier et al. (2006) studied the influence of relative humidity on carbon dioxide permeability in wheat gluten films. They reported that the increasing of the permeability of the gas was observed at 96% relative humidity. This was attributed to the swelling of the polymer matrix with water, allowing chemical interactions to take place between amino acids and the gas. The increase in the water content of the films resulted in promoting the affinity between the gas and the protein matrix, leading to outstanding sorption values at high relative humidity.

4.7. Film additive

Various materials can be incorporated into protein films to influence the mechanical, protective, sensory, or nutritional properties. Plasticizers are additives that are an important class of low molecular weight non-volatile compounds that are widely used in polymer industries. The primary role of such substances is to improve the flexibility and capacity for processing of polymers by lowering the second order transition temperature, the glass transition temperature (T_g). The council of the IUPAC (the International Union of Pure and Applied Chemistry) defined a plasticizer as ''a substance or material incorporated in a material (usually a plastic or elastomer) to increase its flexibility, workability, or distensibility''. These substances reduce the tension of the deformation, hardness, density, viscosity and electrostatic charge of a polymer. At the same time they increase the polymer chain flexibility, resistance to fracture and dielectric constant. Among the other properties also affected are the degree of crystallinity, optical clarity, electric conductivity, fire behavior and resistance to biological degradation (Vieira et al., 2011). The compatibility between polymer and plasticizer is a major effective part of plasticization and various parameters can indicate this feature, including polarity, hydrogen bonding, dielectric constant and solubility parameters (Choi et al., 2004). In addition, another impact factor is solvation, as plasticizers with solubility parameters close to those of the polymer require less energy to fuse or solvate the polymer. The temperature of fusion or gelation is related to the solvation strength of the plasticizer and to the size of its molecule (Rahman & Brazel, 2004).

Generally, two types of plasticizers can be distinguished. Internal plasticization is a result of modifications to the chemical structure of the polymer; external plasticization is obtained by adding an agent which modifies the structure and energy within the three dimensional arrangement of the film polymer (Banker, 1966). In practice the addition of a plasticizer to protein films produces films which are less likely to break and are more flexible and stronger. The reduction of the intermolecular bonds between the polymer chains, and thus the overall cohesion, facilitates elongation of the films and reduces its glass transition temperature. This is manifested by a reduction in the barrier properties to gases, vapors, and film solutes (Banker, 1966). The plasticizers that are most usually used in protein films are mono-, di-, and oligosaccharides (generally glucose syrups or glucose fructose honey), polyols (principally glycerol and its derivatives, polyethylene glycols, and sorbitol) and lipids and its derivatives (fatty acids, monoglycerides and their esters, acetoglycerides, phospholipids, and other emulsifiers). The molecular size, configuration and total number of functional groups of the plasticizer, as well as its compatibility with the polymer, could affect the interactions between the plasticizer and the polymer (Yang & Paulson, 2000).

Jangchud & Chinnan (1999) reported that glycerin was found to be the most suitable plasticizer to incorporate into peanut protein concentrate when compared with sorbitol, propylene glycol and polyethylene propylene glycol or polyethylene glycol. These showed very poor mechanical properties, resulting in unsuccessful permeability tests. The concentration of glycerin between 0.67 to 1.67% of protein did not affect the water vapor permeability and oxygen permeability, but affected the tensile strength and percent elongation properties. Glycerin levels of greater than 0.67% of protein were needed to obtain free-standing film.

Vanin et al. (2005) studied the influence of type and content of plasticizers and the properties of gelatin-based films. Four polyols (glycerol-GLY, propylene glycol-PPG, di-DTG and ethylene glycol-ETG) were tested in different concentrations. The results showed that plasticizer type and content had significant effects on the properties of the resulting films. A higher plasticizing effect on thermal properties was observed with the DTG, followed by PPG, GLY and ETG, principally with low plasticizer content. In relation to the mechanical properties, the GLY showed the greater plasticizing effect and efficiency, but the plasticizing efficiency of DTG on the puncture deformation was also considerable. The mechanical resistance could be related to the glass transition temperature of films. It was not possible to observe the plasticizer effect on the water vapor permeability. However the DTG had shown greater plasticizer efficiency, followed by GLY and ETG, while a counter effect was observed with the addition of PPG.

Andreuccetti et al. (2009) determined the effect of hydrophobic plasticizers derived from citric acid (tributyl citrate, acetyltributyl citrate, triethyl citrate, acetyltriethyl citrate) on the functional properties of gelatin-based films. They found that the addition of ester citrate derivatives in gelatin-based film formulations proved feasible, generating flexible materials. The increasing addition of hydrophobic plasticizers had significantly diminished the tensile strength. Such behavior is similar to that described for films prepared with hydrophilic plasticizers. Regarding water vapor permeability, it was determined that the use of hydrophobic plasticizers caused a slight reduction as observed for gelatin films prepared with glycerol and sorbitol.

5. Improvement of protein-based edible films

5.1. Modification of protein-based edible films by chemical treatment

Proteins are promising biomaterials since films made with them are gas barriers. However, the main limitations of protein films, similar to other biopolymers, are their lack of mechanical strength and poor water vapor barrier because of their hydrophilic nature. A very powerful method to improve water resistance, cohesion, rigidity, and mechanical strength and the barrier properties of films to water is cross-linking. To do this different functional groups of proteins can be used. Protein networks have the ability to interact with a wide range of active compounds. This is done via functional groups acting on their reactive side groups. This has the potential to modify via chemical, physical or enzymatic cross-linking to enhance the functional properties of the films.

Chemical treatments with acid, alkali or cross linking agents have been extensively used to improve film properties. Theoretically, the more protein interaction from chemical treatment occurs then chain structure extends and less permeability and greater tensile strength should be obtained. However, Brandenburg et al. (1993) found that alkaline treatment on soy protein isolate did not affect water vapor permeability, oxygen permeability and tensile strength but alkaline treatment improved the film's appearance (it was clearer, more uniform, and with less air bubble) and elongation at break. Chemical agents used for the covalent cross-linking of protein have included aldehydes such as glutaraldehyde, glyoxal or formaldehyde, and others natural cross linking agents.

Because of the cross-linking used in the protein films, formaldehyde is the simplest of cross linking agents, and has the widest reaction specificity. In addition to the amine group of lysine, it reacts with the side chains of cysteine, tyrosine, histidine, tryptophan, and arginine. Although formaldehyde contains a single functional group, it can react bi-functionally and therefore crosslink. Glutaraldehyde is more specific than formaldehyde; it can react with lysine, cysteine, histidine and tyrosine (Tae, 1983). Protein cross-linking by glyoxal involves lysine and arginine side chain groups (Marquie, 2001) at alkaline pH.

Basically, the reaction between formaldehyde and protein contains two step processes: the first step corresponds to the formation of the methylol compound; and the second one corresponds to the formation of methylene bridges that are cross-links between protein chains. The expected reaction scheme was according to Figure 1.

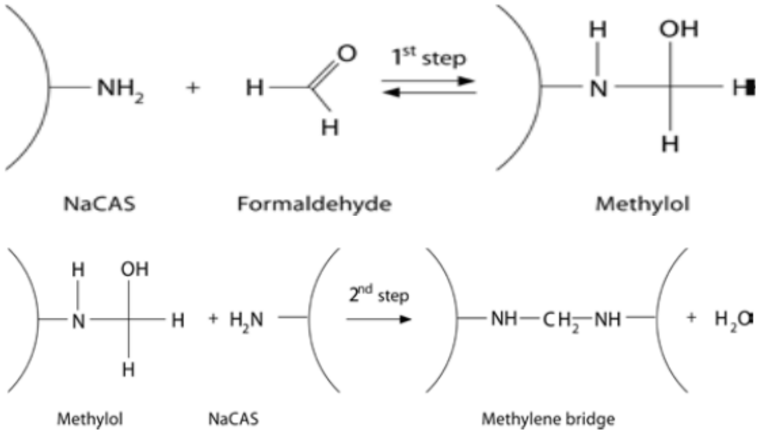


Figure 1. Scheme of cross-linking between formaldehyde and ε-amino groups of protein Source: Gueguen et al. (1998)

Hernandez-Munoz et al. (2004) studied the effect of cross-linking using aldehydes on properties of glutenin-rich films. They reported that the water vapor permeability values of glutenin rich films decreased by around 30% when cross-linking agents such as formaldehyde, glutaraldehyde and glyoxal were incorporated. The highest tensile strength values were obtained using formaldehyde, followed by glutaraldehyde and glyoxal. In addition, the glass transition temperature of cross-linked films shifts to slightly higher values when cross-linking agents were used. Because of this formaldehyde was more efficient than glutaraldehyde and gossypol in cross-linking. The better properties of protein films treated with formaldehyde may be due to the lack of specificity of this chemical with respect to the different amino acid side chain groups. In addition to amines, formaldehyde reacts with sulphydryl, phenolic, imidazolyl, indolyl and guanidinyl groups. Addition, some researchers have reported that the formation of methylene bridges occurred between lysine and tyrosine in formaldehyde-treated of protein (Hernandez-Munoz et al., 2004). However, although highly reactive to aldehyde, they also have a major disadvantage - their toxicity. This must be taken into account when synthesizing biodegradable materials. All of the aldehyde used must be cross-linked in a permanent protein network; the fate of the aldehyde in the environment at the end of the material's life must be considered.

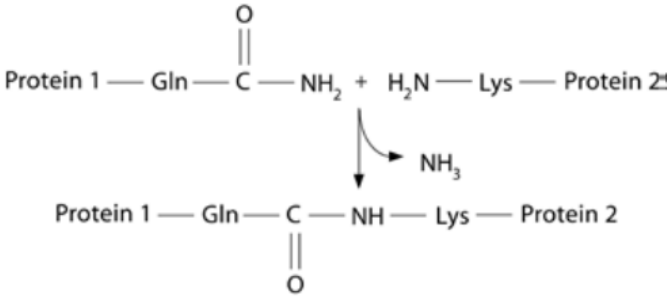
Because of the toxicity of aldehydes, many operators have been trying to use the natural cross linking agents to improve the protein film properties. Orliac et al. (2002) determined the effects of natural cross linking agents (tannins and gallic acid) on the properties of thermo-moulded films produced from sunflower protein isolate. The results showed that the incorporation of tannins and gallic acid resulted in films with higher mechanical properties than for control films, but lower than the films obtained with aldehydes. This was probably because they act through weak interactions rather than covalent bonds in the case of aldehydes. Furthermore, their extremely low volatility at the processing temperature prevents the elimination of the non-bound tannin and gallic acid parts.

Later, Cao et al. (2007) improved the mechanical properties of gelatin films by using ferulic acid and tannic acid. The results showed that the ferulic acid and tannic acid act as natural cross linking agents and had cross-linking effects on gelatin film. The maximal mechanical strength of gelatin film could be obtained when the pH value of the film-forming solution was 7 for ferulic acid as the cross-linked agent, or when the pH value was 9 as for tannic acid. In addition, the properties of gelatin films treated by tannic acid could become better after being stored for more than 90 days, while the storage time had little effect on ferulic acid-modified films. The reason may be because tannic acid could cross link with gelatin later step-by-step with time during drying and storage.

5.2. Modification of protein-based edible films by enzymatic treatment

Protein- based edible films have good barrier characteristics against gas, organic vapor and oil as compared to synthetic films. However, the weak mechanical characteristics and the high water permeability of protein films limit their application as a packaging material. Many studies have been carried out in an attempt to improve the performance of protein-based edible films. The technique for improving the functionality of protein-based edible films is to modify the polymer network through the cross linking of the polymer chains. One effective technique for improving the barrier properties and mechanical strength of protein-based edible films is a cross linking technique using enzymatic methods. Some enzymes that have been used for cross linking proteins include transglutaminase (TGase; EC.2.3.2.13), lipoxygenase, lysyl oxidase, polyphenol oxidase and peroxidase. However, transglutaminase is a kind of enzyme which can catalyze the covalent cross linking reactions between proteins to form high molecular weight (MW) biopolymers. De Jong & Koppelman (2002) reported that transglutaminase catalyzes acyltransfer reactions between λ-carboxyamide groups of glutamine residues (acyl donor) and ε-amino groups of lysine residues (acyl acceptor), resulting in the formation of ε-(λ-glutaminyl) lysine intra and intermolecular cross-linked proteins. The reaction catalyzed of glutamyltransferases is shown in Figure 2 (Yee et al., 1994)

Polymerization using transglutaminase has been investigated with various protein sources including casein, soy proteins and gelatin, where different responses in gel strength were dependent on the reaction conditions and on the different protein sources (Sakamoto et al., 1994). The increase in gel strength of proteins submitted to the action of transglutaminase depended on the order and intensity by which the enzyme produced cross links, and the extent to which these new covalent linkages could impede the 'physical' cross-linkages occurring during renaturation and formation of the triple helix during gel formation (Babin & Dickinson, 2001). Larre et al. (2000) reported that transglutaminase was effective in introducing covalent bonds into films obtained from slightly deamidated gluten. The establishment of these covalent bonds induced the formation of polymers of high molecular weight that were responsible for the greater insolubility of the treated films but a reduced surface hydrophobicity. Mechanical properties showed that the addition of covalent bonds by the use of transglutaminase increased the film's integrity and heavy-duty capacity as well as its capacity to stretch.



$$\text{Protein 1}-\text{Gln}-\overset{\overset{\displaystyle O}{\|}}{C}-NH_2 + H_2N-\text{Lys}-\text{Protein 2} \xrightarrow{-NH_3} \text{Protein 1}-\text{Gln}-\underset{\underset{\displaystyle O}{\|}}{C}-NH-\text{Lys}-\text{Protein 2}$$

Figure 2. The reaction catalyzed of glutamyltransferases Source: Yee et al. (1994)

Generally, the cross-linkage by transglutaminase improves the tensile strength of protein films, while it decreases the elongation at break and solubility properties. In some cases, such as with isolated soy protein and deamidated gluten films, the transglutaminase treatment also significantly increased the surface hydrophobicity of films (Tang et al., 2005).

However, there are many problems involved in this cross linking technique that have to be investigated before it is commercially applied to films. For example, it is uncertain that this cross-linking by enzyme can improve the properties of films cast from various food proteins. Furthermore, for a certain proteins there are optimal processing parameters to obtain the best effects in the improvement. The improvement in the properties of protein-based edible films by using enzyme seems to be dependent upon the types of substrate protein and some processing parameters, such as the amount of enzyme applied. Thus it is expected that the improvement in the properties of protein-based edible films by enzyme treatment is also affected by the enzyme concentration, since coagulation or aggregation decreases the mechanical properties of protein-based edible films. Jiang et al. (2007) reported that the properties of soy protein films, especially the tensile strength and the hydrophobicity, could be modified by the transglutaminase. However, their work indicated that the modification of properties of soy protein films by transglutaminase was, to a various extent, dependent upon many processing parameters. These included the concentration of enzyme, the condition of the film-forming solutions and the air drying temperature. The influence of these processing parameters could account for the aggregation of soy protein films induced by transglutaminase. Thus, the improvement of the tensile strength of soy protein films by transglutaminase could be achieved by inhibiting or delaying the occurrence of this kind of aggregation.

5.3. Modification of protein-based edible films by irradiation treatment

Although proteins are known for showing good film forming abilities, protein films have rather moderate barrier properties. A need exists therefore to search for new substances and processes in order to obtain better products. Inducing cross linking through using irradiation was found to be an effective method for the improvement of both barrier and mechanical properties of protein-based edible films. Generally, irradiation affects proteins by causing conformational changes, oxidation of amino acids, rupture of covalent bonds, and the formation of protein free radicals (Cheftel et al., 1985). Proteins can be converted to higher molecular weight aggregates through the generation of inter-protein cross-linking reactions, hydrophobic and electrostatic interactions, and the formation of disulfide bonds (Davies & Delsignore, 1987). For example, the hydroxyl and superoxide anion radicals that are generated by the radiation of film-forming solutions can modify the molecular properties of proteins. This can result in the alteration of the protein films by covalent cross-linkages formed in the protein solution after irradiation (Garrison, 1987).

The formation of high molecular weight aggregates was negligible at the low-dose range, but increased significantly with higher doses. Irradiation treatment for the improvement protein films, such as gamma irradiation, has been extensively used to modify protein. Ouattara et al. (2002) used gamma irradiation cross-linking to improve the water vapor permeability and the chemical stability of milk protein films. The results showed that gamma irradiation significantly ($p< 0:05$) reduced water vapor permeability and increased resistance to microbial and enzymatic biodegradation. An increase in the concentration of high molecular weight proteins in the film forming solution was also observed. They pointed out that two hypotheses may explain the effect on gamma irradiation: (i) The participation of more molecular residues in intermolecular interactions when used in proteins with different physicochemical properties. (ii) The formation of inter- and/or intra-molecular covalent cross-links in the film-forming solutions.

Ciesla et al. (2004) also investigated the effect of gamma irradiation on the physical properties of milk proteins. They found that the viscosity of the irradiated proteins film solutions was increased as compared to the control films. This was because the gamma irradiation developed a ''fine-stranded'' structure of the protein gel. The creation of the better ordered gels after irradiation corresponds well to the rearrangement of the cross linked β-phase (accompanied by the reorganization of a periodic phase). Using gamma irradiation therefore causes more improvement in well organized β-conformation than non irradiated milk protein films. In addition, the presence of the better ordered protein conformations in gels obtained from irradiated solutions leads to production of more ''crystalline'' films. These films are characterized by improved barrier properties and mechanical resistance and higher rigidity than those prepared from the non irradiated solutions.

Lee et al. (2005) reported that the gamma irradiation treatment of the gluten film solutions caused the disruption of the ordered structures of the protein molecules. It changed tensile strength, elongation at break and water vapor permeability. They observed that the increased tensile strength of gluten films suggests that cross-linking occurred as a result of gamma irradiation treatment. The increase in tensile strength was possibly caused by the increase of the aggregation of polypeptide chains under experimental condition in this study. It can be assumed that the formation of high molecular weight proteins, aggregated from cleaved polypeptide chains by gamma-irradiation, may be responsible for the reduction of water vapor permeability by reducing the rate of diffusion through the film.

Soliman et al. (2009) studied the influence of gamma irradiation on the mechanical and water barrier properties of corn protein-based films. This study showed that gamma irradiation treatment has potential for modifying the physicochemical properties of zein based films, particularly the water barrier properties. Through the formation of high molecular weight proteins from disaggregated protein particles and cleaved polypeptide, chains can be generated by gamma irradiation. The linkages formed can reduce the absorption of water molecules into the film and the diffusion through the film.

5.4. Modification of protein-based edible films by combination with hydrophobic materials

Generally protein films had good mechanical properties. However, the hydrophilic nature of protein films causes them to be less effective moisture barriers. Conversely, lipid films are good moisture barriers, but they are usually opaque, relatively inflexible, unstable (they tend to be rancid) and taste like waxy. Improved film performances are obtained with a multi-component system where proteins form a continuous and cohesive network, and the lipids provide moisture barrier properties. Lipids can form a layer over the hydrocolloid matrix (bilayer films) or can be dispersed into the matrix (emulsified films). In practice emulsified films have received more interest than bilayer films. Two models have been proposed to describe transfer through emulsified films. Ukai et al. (1976) proposed the 'microvoid model' and suggested that the mass transfer of gases and vapors occurs through microvoids. These are formed between the micro particles of the hydrophobic material and the hydrocolloid matrix during emulsion drying. Krochta et al. (1990) proposed an alternative model, by the 'micro pathway model'. This attributes mass transfer through the high polymer matrix itself. This can occur because proteins are often quite compatible with moisture and gases and can offer little resistance to their transmission.

The addition of lipids to protein films may interfere with polymer chain-to-chain interactions and/or provide flexible domains within the film. Because of their lack of cohesive structural integrity they could also affect the mechanical properties of the protein film. Pérez-Gago & Krochta (2000) reported that the type and content of the lipids were important in controlling the water vapor permeability of protein emulsion films but also had a negative effect on their mechanical properties. Consequently, the components of protein films should be carefully selected and in accord with the final application of the protein films. A protein film should be resistant in order to withstand manipulation during its application and to maintain its integrity and also its barrier properties.

McHugh & Krochta (1994) produced whey protein and lipid emulsion films and found that the water vapor permeability of the films was reduced through the incorporation of lipids. Fatty acid and beeswax emulsion films exhibited very low water vapor permeability. Gontard et al. (1994) also reported that beeswax was the most effective lipid to improve the moisture barrier of films prepared from wheat gluten. Combining wheat gluten protein with diacetyl tartaric ester monoglycerides reduced water vapor permeability, increased tensile strength and maintained transparency. Anker et al. (2002) produced composite whey protein isolated lipid films (laminate and emulsion films) to improve barriers against water vapor. The laminated whey protein lipid film decreased the water vapor permeability 70 times compared with the whey protein film. The water vapor permeability of the emulsion films was half the value of the whey protein isolated film. Regarding the mechanical properties, the results showed that the lipid functioned as an apparent plasticizer by enhancing the fracture properties of the emulsion films. Bertan et al. (2005) incorporated Brazilian elemi (highly hydrophobic resinous oil) into a gelatin film using a blend of palmitic and stearic acids. They evaluated the physicochemical characteristics of the resulting films, all of which contained triacetin as the plasticizer. For films with added acids, the blend and the elemi presented better water vapor barrier properties as compared to the gelatin/triacetin film. However, the mechanical resistance decreased with the addition of the lipids and the opacity and soluble matter increased.

5.5. Modification of protein-based edible films by combination with synthetic polymers

Generally protein-based edible films show an excellent oxygen barrier property at low to intermediate relative humidity as well as fairly good mechanical properties. However, their barrier against water vapor is poor due to their hydrophilic nature (Avena-Bustillos & Krochta, 1993). Many researchers have focused on improving the film properties of protein-based edible films, particularly their mechanical and water vapor barrier properties, by the modification of the films. However, despite the improvement in protein film properties, their physical, thermal, and mechanical properties are still not satisfactory and these present difficulties in many applications. Existing composite films containing layers of different film materials may be needed. Therefore, there is an increasing interest in the development of protein-based edible films for packaging materials. These must have suitable properties for application and can be disposed of after use in an economically and ecologically acceptable way.

Oxygen-barrier layers in food packaging materials typically consist of expensive synthetic barrier polymers including low density polyethylene (LDPE) and polypropylene (PP). These are commonly used in the form of coextruded or laminated films and coatings. It is important to choose the proper polymer matrix as the mechanical and barrier properties can be improved to a great extent by forming the composite plastic films on conventional protein films. To obtain fundamental data on these composite structures further investigations are needed. These should focus on both the optimization of coating formulations used to prepare biopolymer-coated plastic films, and the evaluation of their physical properties.

Hong & Krochta (2006) reported that smooth and transparent oxygen-barrier coatings based on whey proteins can be formed on common plastic films such as polyethylene and polypropylene. The resulting whey protein-coated plastic films with glycerol as a plasticizer have excellent oxygen-barrier properties at low to intermediate RH, and are comparable to synthetic oxygen barriers. These coated films also have good visual quality and adhesion between the coating and the substrate. Because of this, it is suggested that whey protein coatings could be a new biopolymer oxygen barrier. These have great potential for partly replacing existing expensive synthetic barrier polymers in various composite packaging structures.

Lee et al. (2008) characterized the protein-coated polypropylene films as a new composite structure for application in food packaging. The composite structure of polypropylene film coated with three kinds of proteins (soy protein isolate: SPI, whey protein isolate: WPI, corn zein: CZ) could be obtained by a simple casting method. High glossy surfaces were observed on the films coated with WPI and CZ. Proteins exerted a noticeable effect on color of the coated films. WPI coated films also showed greater transparency and tensile strength than the other coated films. These results suggested that WPI coatings with a proper plasticizer possess excellent visual and mechanical characteristics and have great potential for application in food packaging systems.

Tihminlioglu et al. (2010) determined the water vapor and oxygen-barrier performance of corn–zein coated polypropylene films. They found that significant improvements in water vapor and oxygen barrier properties of uncoated polypropylene films were obtained with corn–zein coating. The water vapor permeability of the coated films decreased significantly with increasing corn–zein concentration. The application of plasticized corn–zein coating on polypropylene films showed nearly a three times reduction in oxygen permeability. The high water vapor and oxygen-barriers were obtained for films coated with coating formulation consisting of higher amounts of corn–zein.

6. Application of protein-based edible films

Protein-based edible films offer alternative packaging without adversely affecting the environmental costs. However, edible films are not meant to totally replace synthetic packaging or to limit moisture, aroma and lipid migration between food components where traditional packaging cannot function. For instance, protein-based edible films can be used for versatile food products to reduce loss of moisture, to restrict absorption of oxygen, to lessen migration of lipids, to improve mechanical handling properties, to provide physical protection, or to offer an alternative to commercial packaging materials (Kester & Fennema, 1986).

Protein- based edible films have impressive gas barrier properties compared with those prepared from lipids and polysaccharides. When they are not moist, the O_2 permeability of soy protein-based film was 500, 260, 540 and 670 times lower than that of low-density polyethylene, methylcellulose, starch and pectin respectively (Cuq et al., 1998). In addition, the mechanical properties of protein-based edible films are also better than those of polysaccharide and fat-based films. This is because proteins have a unique structure which confers a wider range of functional properties, especially a high intermolecular binding potential. Protein- based edible films may be able to partially replace some of the conventional synthetic packaging materials used to preserve and protect foods. These protein-based edible films should not be used alone, since contamination during food handling could occur, but would be used to wrap foods inside a secondary synthetic package during food distribution and storage. The wraps could also be used in the home to cover leftovers in the refrigerator, peeled fruit mixtures or as a sandwich bag for lunch. Because such wraps are biodegradable and may even be eaten, they are not harmful to the environment. This attribute could also reduce waste disposal costs (McHugh et al., 1996).

Several researchers have studied the application of protein-based edible films in food use and an excellent review of these is given by Gennadios et al. (1994). They reviewed the applications of several protein-based edible films, such as corn zein on nut and fruit products, casein emulsion film on fruit, and whey protein films on fruit products. However, the application of edible films from water-soluble fish protein in surimi wash-water was not mentioned in their review. One of the potential uses of protein films is in reducing lipid oxidation due to the film's excellent barrier properties. Stuchell & Krochta (1995) used whey protein isolate and acetylated monoglyceride to maintain the quality of frozen king salmon and found a delay in the lipid oxidation onset and a reduction in moisture loss rate. Herald et al. (1996) used corn zein with an antioxidant and emulsifier to maintain the quality of cooked turkey. They found that dipping it in corn zein resulted in very dry products in terms of sensory evaluation. However, corn zein with an antioxidant and emulsifier reduced hexanol after 3 days when compared with PVDC films. Schou et al. (2005) used the sodium caseinate films for wrapping bread. They found that bread samples wrapped with single or double layered sodium caseinate film had the lower value in the compression test among the unwrapped samples. These films prevented the hardening of the bread to the same extent as the polyvinyl chloride films after 3 h storage. After 6 h storage, the sodium caseinate films were not as effective as synthetic film but still reduced the hardening of the bread relative to the unwrapped samples.

7. Conclusion

The protein-based edible films have advantages, including their use as edible packaging materials, over synthetic films. In addition, these films have impressive gas barrier properties compared with those prepared from lipids and polysaccharides. The mechanical properties of protein-based edible films are also better than those of polysaccharide and fat-based films because proteins have a unique structure. Technically, the properties of protein-based edible films depend on the type of proteins, polymer chemistry, processing conditions and the additives used. However, protein-based edible films still show poor both water vapor resistance and mechanical strength in comparison with synthetic polymers and this limits their application in food packaging. Their properties could be improved by chemical and enzymatic methods, by combining them with hydrophobic material or some synthetic polymers or by using a physical method. The resulting film properties depend on the methods of modification and conditions. The chemical and enzyme modifications were efficient in increasing mechanical and water barrier properties. However, modification with chemical compounds, especially aldehydes, would cause toxicity. This must be a matter for concern in the application of chemical modification.

Edible protein-based edible films combination with hydrophobic materials and synthetic polymer can result in better functionality than films produced with only proteins, especially with respect to their mechanical and barrier properties. Irradiation was found to be an effective method for the improvement of both barrier and mechanical properties of protein-based edible films. Protein-based edible films show impressive gas barriers; hence protein films would be a suitable food packaging material for preventing the growth of aerobic microbial and lipid oxidation in lipid enriched foods.

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