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Review Article
Edible protein films: properties enhancement

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Abstract: Edible films can be prepared from protein, polysaccharide and lipid materials. Among them, protein-based edible films are the most attractive. 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 (based on 20 different monomers) which confers a wider range of functional properties, especially a high intermolecular binding potential. However, the poor water vapor resistance of protein films and their lower mechanical strength in comparison with synthetic polymers limit their application in food packaging. Hence, improvement of edible protein film properties has been investigated to seek suitable applications. The objective of this review is to provide a detailed enhancement of the properties of edible protein film through using various methods such as modifying the properties of protein by chemical and enzymatic methods, combining them with hydrophobic material or some polymers, or using a physical method. These methods focus primarily on improving the mechanical strength and moisture barrier properties.

Key words: Edible protein films, properties enhancement, chemical treatment, enzymatic treatment, hydrophobic combination, Irradiation

Introduction

Currently there has been a renewed interest in edible film made from renewable and natural polymer such as protein, polysaccharide and lipids. Edible polymer films are not meant to totally replace synthetic packaging film and limit moisture, aroma and lipid migration between food and aroma, and lipid migration between food components, where traditional packaging cannot be used. For instance, 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 the commercial packaging materials. The films can enhance the organoleptic properties of packaged foods provided that various components (such as flavorings, colorings and sweeteners) are used. The films can be used for individual packaging of small portions of food, particularly products that are currently not individually packaged for practical reasons. These include pears, beans, nuts and strawberries. In 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 edible films could be their use in multilayer food packaging materials together with non edible films. In this case the edible films would be the internal layers in direct contact with food materials (Murray and Luft, 1973; Kester and Fennema, 1986; Nelson and Fennema, 1991). Natural polymers or polymers derived from natural products, like food protein, offer the greatest opportunities since their biodegradability and environmental compatibility are assured (Krochta and De Mulder-Johnston, 1997). In addition, films made from protein can supplement the nutritional value of the food (Gennadios and Weller, 1990). 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 (based on 20 different monomers) which confers a wider range of functional properties, especially a high intermolecular binding potential (Cuq *et al*., 1995). Protein-based edible films can form bonds at different positions and offer high potential for forming numerous linkages (Ou *et al*., 2005). However, the poor water vapor resistance of protein films and their lower mechanical strength in comparison with synthetic polymers limit their application in food

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packaging. Many approaches exist to improve the barrier properties of edible protein films, such as by modifying properties of protein by chemical and enzymatic methods, combining with hydrophobic material or some polymers, or by using a physical method. The methods primarily focus on improving the mechanical strength and moisture barrier properties.

Modification of edible protein films by chemical method

Chemical treatments with acid, alkali or cross-linking agents have been extensively used to improve the properties of films. Hydrolyzed protein results in greater solubility at high pH and high temperature (Bain *et al*., 1961). Guilbert (1986) reported that denatured protein forms less flexible and transparent but more moisture-resistant films. Theoretically, the more protein interaction from chemical treatment such as alkaline or acid modification would occur with extended chain structures; 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. However, alkaline treatment improved a film's appearance (making it clearer, more uniform, with less air bubbles) and elongation at breaking points. 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. Chemical agents used for covalent cross-linking of protein have included formaldehyde, glyceraldehyde, glyoxal and others (Orliac, *et al*., 2002; Hernandez-Munoz *et al*., 2004). Formaldehyde is the simplest of cross-linking agents and has the broadest reaction specificity. Although formaldehyde contains a single functional group, it can react bi-functionally and can 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. The expected reaction scheme (Gueguen *et al*., 1998) was according to Figure 1. The reaction between formaldehyde and protein is a two step process: the first step corresponds to the formation of the methylol compound and the second one corresponds to the formation of methylene bridges that is cross-links between protein chains.

Hernandez-Munoz *et al*. (2004) reported that addition of cross-linking agents to the film-forming solution of glutenin-rich films with glutaraldehyde (GTA), glyoxal (GLY) and formaldehyde (FA)

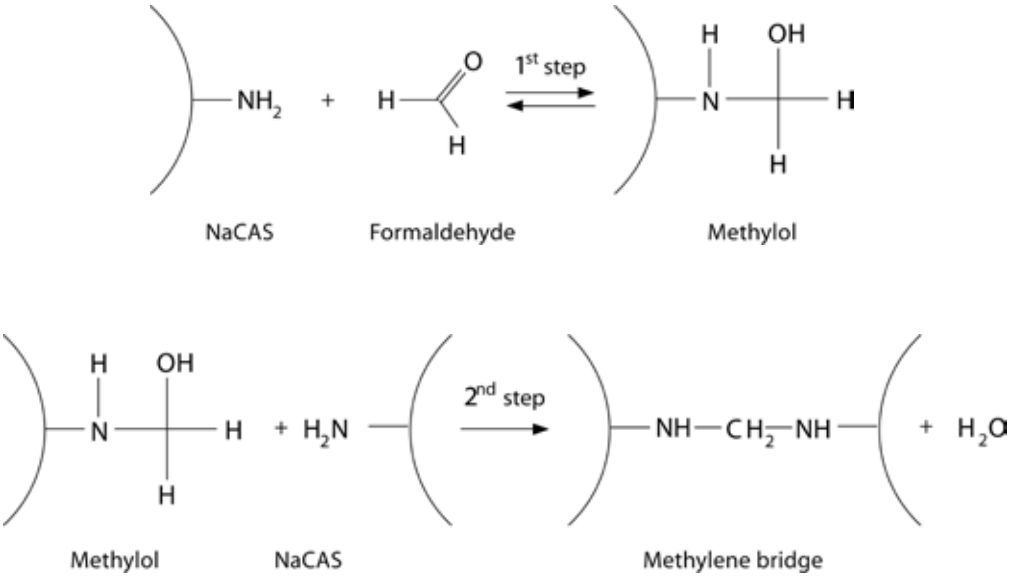

Figure 1. Scheme of cross-linking between formaldehyde and ε-amino groups of protein (Source: Gueguen *et al*., 1998)

Table 1. Tensile strength (TS) and percentage of elongation at break (%E) for glutenin-rich films plasticized with 33% glycerol (w/w) at different concentrations of cross-linker

Treatment	TS (MPa)	%E
Control	5.9 + 0.8	260 + 17
Formaldehyde (%)		
2	13.8 + 1.8	100 + 27
4	13.4 + 0.9	96 + 22
8	13.1 + 1.5	96 + 18
Glutaraldehyde (%)		
2	8.6 + 1.1	131 + 32
4	9.6 + 2.3	165 + 22
8	9.1 + 0.8	164 + 24
Glyoxal (%)		
2	7.2 + 0.9	206+39
4	7.7 + 1.3	209 + 33
8	7.9 + 0.9	199 + 37

Source: Adapted from Hernandez-Munoz *et al.* (2004)

enhances the water barrier properties of the films, an increase in the resistance to breakage, and decreased film deformability (Table 1). The formation of more resistant films suggests the occurrence of new covalent bonds between glutenin proteins via chemical reaction through FA, GTA and GLY and amino acid side chain reactive groups. The FA is the most effective cross-linker in terms of these properties. Formaldehyde is a low molecular weight molecule and could easily migrate between the protein chains and establish new covalent bonds with the Lys, Cys and His groups of the proteins (Gallieta *et al*., 1998). IN addition, the higher TS values for films treated with formaldehyde can 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 (Fraenkel-Conrat and Olcott, 1948a, b). Blass *et al*. (1965) also reported the formation of methylene bridges between lysine and tyrosine in formaldehyde-treated tetanus and diptheria. Nevertheless, due to the toxicity of the chemicals used as cross-link glutenins, future research should take place in order to analyze the aldehyde residues remaining in the film and their migration in the event of these materials being used in direct contact with foods. Additional rigorous studies into the use of non-toxic alternative cross-linkers need to be undertaken.

Modification of edible protein films by enzymatic treatment

Many studies have been carried out in an attempt to improve the performance of protein films. The alternative to improving protein film functionality is to modify the polymer network through the cross-linking of the polymer chains. An enzyme that has received extensive recent attention for its capacity to cross-link protein is transglutaminase. Transglutaminase (Tgse, protein-glutamine γ-glutamyl transferase, E.C.2.3.2.13) catalyzes acyl-transfer 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 (De Jong and Koppelman, 2002). The reaction catalyzed of glutamyltransferases is shown in Figure 2 (Lee *et al*., 1994). The formation of the cross-linking does not reduce the nutritional quality of the food as the lysine residue remains available for digestion (Seguro *et al*., 1996; Yokoyama *et al*., 2004). In the past, the limited availability and high cost of transglutaminase limited its application. Nowadays, transglutaminase from a microbial source that is significantly lower in price is commercially available from Ajinomoto Inc. (Kawasaki, Japan). This makes its potential use as a cross-linker in films feasible.

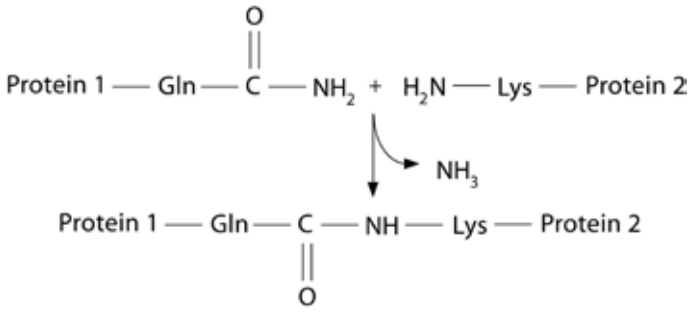

Figure 2. The reaction catalyzed of glutamyltransferases (Source: Yee *et al*., 1994)

Polymerization using transglutaminase has been investigated with various protein sources including a-casein, soy proteins and gelatin, where different responses in gel strength were dependant 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 and Dickinson, 2001). Mahmoud and Savello (1993) investigated the production of whey protein films using transglutaminase as the catalytic cross-linking enzyme. Transglutaminase could catalyze the covalent polymerization of whey protein. However, the effect of using transglutaminase on a film's permeability was not available. Stuchell and Krochta (1994) studied enzymatic treatments on edible soy protein films. The results showed that treatment with horseradish peroxidase provided no further improvement in water vapor permeability, but increased tensile strength and protein solubility and decreased elongation. Yildirim *et al*. (1996) prepared biopolymer from crosslinking whey protein isolate and soybean by transglutaminase. The biopolymer showed excellent stability, thus using polymers should result in the formation of better water moisture barrier films. Larre *et al*. (2000) showed 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 (Table 1). Babin and Dickinson (2001) observed that treatment with transglutaminase could present both positive and negative effects on the strength of gelatin Types A and B. This depended on the order in which the cross-linkages were formed, and whether before or after the formation of junction zones induced by the cooling of the solution to temperatures below 35oC. Oh *et al*. (2004) used transglutaminase to produce whey protein or casein films by incorporating zein hydrolysate. These films exhibited higher elongation values but lower tensile strength without any significant effects on the water vapor permeability of the protein films. Transglutaminase catalysed cross-link reactions between casein and zein molecules which resulted in improving the flexibility of the films. The heterologous cross-linking between two proteins by transglutaminase probably depends on the thermodynamic compatibility of mixing of the substrate proteins at the enzyme's active site (Han and Damodaran, 1996).

Modification of edible protein films by combination with hydrophobic materials

The barrier properties of bio-polymeric films are important parameters when considering a suitable barrier for use in foods and food packaging. Protein films are generally good barriers against oxygen at low and intermediate relative humidity (RH) and have good mechanical properties, but they are poor barriers against water vapor. Being a poor barrier is due to their hydrophilic character. In many applications, a better barrier against water vapor is preferable since low levels of water activity must be maintained in low-moisture foods to prevent texture degradation and to minimize deteriorative chemical and enzymatic reactions (Kester and Fennema, 1986). Therefore, the hydrophobic properties of lipids are exploited for their great water barrier properties, and especially high melting point lipids, such as beeswax or carnauba wax (Shellhammer and

Krochta, 1997; Morillon *et al*., 2002). A composite film made of a protein and a lipid can be divided into laminates (in which the lipid is a distinct layer within or atop the biopoly-meric films) and emulsions (in which the lipid is uniformly dispersed throughout the biopolymeric film). Both the laminate and emulsion films offer advantages. The laminate films are easier to apply with regard to the temperature, due to the distinct natures of the support matrix and lipid (Koelsch, 1994). During the casting of the lipid onto the protein film, the temperatures of the film and lipid can easily be controlled separately. When producing the emulsion films, the temperature of the emulsion must be above the lipid-melt temperature but below the temperature for solvent volatilization of the structural network. The main disadvantage of the laminated films, however, is that the preparation technique requires four stages; two casting and two drying stages. This is why the laminated films are less popular in the food industry despite their being good barriers against water vapor (De-beaufort and Voilley, 1995). The preparation of the emulsion films requires only one casting and one drying stage, but the finished films are still rather poor barriers against water vapor, since the water molecules still permeate through the non-lipid phase. The reason for this is the non-homogeneous distribution of lipids. However, they have the advantages of exhibiting good mechanical resistance, and to require a single step during the manufacture and application process, against one step per layer for multilayer films. It has been shown that for emulsion-based films the smaller the lipid globule size is, and the more homogeneously distributed they are, the lower the water vapor permeability (McHugh and Krochta, 1994; Debeaufort and Voilley, 1995; Perez-Gago and Krochta, 2001). Many researchers have examined the water vapor permeability and mechanical properties of composite films made from proteins with added lipids. For example, composite protein-lipid films had lower water vapor permeability values than control protein films from caseinates (Avena-Bustillos and Krochta, 1993), whey protein (McHugh and Krochta, 1994a,b; Banerjee and Chen, 1995; Perez-Gago and Krochta, 1999; Berntsen, 2000), zein (Weller *et al*., 1998), and wheat gluten (Gennadios *et al*, 1993; Gontard *et al*., 1994). The reduced migration of moisture in bicomponent foods has also been studied. Ukai *et al*. (1976) patented the use of protein-lipid emulsion (caseinate-based emulsion) for coating agricultural products. Guilbert (1986) developed bilayer emulsion protein based films using casein or gelatin, and stearic-palmitic acid and canauba wax. These films showed good water barrier properties, but poor mechanical properties and residual waxy taste. McHugh and Krochta (1994a) developed whey protein-lipid emulsion films and found that the water vapor permeability of films was reduced through lipid incorporation. Fatty acid and beeswax emulsion films exhibited very low water vapor permeability. Gontard *et al*. (1994) reported that beeswax was the most effective lipid to improve moisture barrier of films prepared from wheat gluten. Combining wheat gluten protein with diacetyl tartaric ester monoglycerides reduced water vapor permeability, increasing tensile strength and maintained transparency. Park *et al*. (1994) reduced water vapor permeability of corn zein by lamination with methylcellulose and zein-fatty acid (lauric acid, plamitic or blended of stearic acid and palmitic acid). Shih (1994) found that alkylated complexes (protein-propylene glycol alginate) showed better film making properties and good stability in water but the non-edibility of the specific reducing agent sodium cyanoborohydride may limit its use with food. Anker *et al*. (2002) produced composite whey protein isolated-lipid films (laminate and emulsion films) to improve the barrier against water vapor. The laminated whey protein-lipid film decreased the water vapor permeability 70 times compared with the WPI 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 the Brazilian elemi (highly hydrophobic resinous oil) into a gelatin film using a blend of palmitic and stearic acids, and 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.

Modification of edible protein films by irradiation

Although proteins are known for having good film forming abilities, protein films have rather moderate barrier properties. It is therefore necessary to search for new compositions and processes in order to obtain better products. To improve the functional properties of protein films, cross-linking agents or ionizing radiation have been tried (Yamada *et al*., 1995; Rhim

et al., 1999; Vachon *et al*., 2000). Gamma-irradiation affects proteins by causing conformational changes, oxidation of amino acids, and the rupture of covalent bonds and formation of protein free radicals (Cheftel *et al*., 1985). Chemical changes in the proteins that are caused by gamma-irradiation are fragmentation, cross-linking, aggregation, and oxidation by oxygen radicals that are generated in the radiolysis of water (Schuessler and Schilling, 1984; Filali-Mouhim *et al*., 1997; Cho and Song, 2000). For example, the hydroxyl and super oxide anion radicals that are generated by radiation of film forming solution could modify the molecular properties of the proteins, which results in the alteration of protein films by covalent cross-linkages formed in protein solutions after irradiation (Garrison, 1987). Cross-linking induced through using gamma irradiation was found to be an effective method for the improvement of both barrier and mechanical properties of the edible films and coatings based on protein. Indeed, the irradiation of aqueous protein solutions generate hydroxyl radicals (*OH) that produce stable compounds like bityrosine (Brault *et al*., 1997). Lacroixa *et al*. (2002) reported that gamma-irradiation was efficient for inducing cross-links in whey, casein and soya proteins edible films. The cross-linking reactions affected moderately the protein structure. Modification of protein conformation could be a result of irradiation treatment, inducing modified structures that are more ordered and more stable. 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. 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; and (ii) the formation of inter- and/or intra-molecular covalent cross-links in the film-forming solutions (Ouattara *et al*., 2002). Lee *et al*. (2004) stated that the gamma irradiation of soy protein isolated solutions caused the disruption of the ordered structure of the soy protein isolated molecules, as well as degradation, cross-linking, and aggregation of the polypeptide chains, which all results in decreased water vapor permeability by 13%. In addition, the tensile strength of the soy protein isolated films doubled through gamma-irradiation. The results clearly indicate that the molecular properties of soy protein isolated film forming solutions could be altered by gamma irradiation. Therefore, gamma irradiation can be a useful tool as a cross-linking agent to improve the functional properties of soy protein isolated films. Lee *et al*. (2005) studied the effect of gamma-irradiation on the physiocochemical properties of gluten films.

Conclusion

The properties' enhancement characteristics of edible protein can be applied with chemical and enzymatic methods, combining with hydrophobic material or some polymers or using a physical method. The resulting film properties depend on modification methods and conditions. The enzyme and chemical modifications were efficient in lowering water vapor permeability, with the former showing itself more efficient as compared to the native film, but neither the enzyme treatment nor the glyoxal chemical treatment were efficient in improving the mechanical properties, although treatment with formaldehyde resulted in a significant increase in tensile strength. Nevertheless, due to the toxicity of the chemicals used to cross-link protein, application of chemical modification must raise the issue of the aldehyde residues remaining in the film and their migration in the event of these materials being used in direct contact with foods. Composite edible protein films in combination with lipids can result in better functionality than films produced with only proteins, especially with respect to their barrier properties. Of the lipids, waxes produce the best water vapor barrier properties, but produce fragile and/or brittle films. Gamma-irradiation affects proteins by causing conformational changes, oxidation of amino acids, and rupture of covalent bonds and formation of protein free radicals. Chemical changes in the proteins that are caused by gamma-irradiation are fragmentation, cross-linking, aggregation, and oxidation by oxygen radicals that are generated in the radiolysis of water. For example the hydroxyl and super oxide anion radicals that are generated by radiation of film forming solution could modify the molecular properties of the proteins, which results in the alteration of protein films by covalent cross-linkages formed in protein solution after irradiation. Using gamma irradiation to induce cross-linking was found to be an effective method for the improvement of both barrier and mechanical properties of the edible films and coatings based on protein.

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