CHAPTER 1

INTRODUCTION

Natural biopolymers have been paid increasing attention for manufacturing edible or biodegradable materials owing to their biocompatibility (Irissin-Mangata et al., 2001). Protein-based films have been used commercially for packaging or coating. A variety of proteins, both plant and animal origins, can be used as film-forming agents. Surimi is the concentrated myofibrillar proteins prepared from fish mince that is washed with water and blended with cryoprotectants (Park and Morrissey, 2000). Surimi has been known to exhibit the gelling property, which makes it useful as a food base in seafood analogue. Furthermore, film formation is another functionality of surimi or fish protein (Shiku et al., 2003; Cuq et al., 1995; Paschoalick et al., 2003). Protein are thermoplastic heteropolymers containing both polar and non-polar amino acids, which are able to form the numerous intermolecular linkages. Generally, globular proteins must be denatured by heat, acid, base and/or solvent to form more extended structures that are required for film formation (Krochta, 1997). Solubilizing process directly affects the property of film. Fish myofibrillar protein film showed the different properties, depending on the pH of film-forming solution (Shiku et al., 2003). Protein films are quite stiff and brittle due to the extensive interactions between protein chains through hydrogen bonding, electrostatic forces and hydrophobic interaction (Krochta, 2002). Normally, low-molecular-weight plasticizers must be added in order to improve film flexibility by reducing those interactions. Plasticizers commonly used in protein films include

mono-, di-, and oligosaccharides, polyols and lipids (Avena-Bustillos and Krochta, 1993; Shellhammer and Krochta, 1997). Due to the high content of hydrophilic components, protein film is generally a poor barrier to moisture (Kim and Ustunol, 2001). The addition of lipids to improve the water barrier property may cause the decrease in mechanical property of film. Protein films can be strengthened when crosslinkers such as aldehyde as well as transglutaminase were used (Mahmoud and Savello ,1993; Yidrrim and Hettarachchy ,1998; Marquie, 2001).

Thailand is one of the largest surimi producers in Southeast Asia. Bigeye snapper (*Priacanthus* spp.), threadfin bream (*Nemipterus* spp.), lizardfish (*Saurida* spp.), goat fish (*Parupeneus* spp.) are commomly used as the important raw materials (Benjakul et al., 2003). So far, the information concerning the properties of film produced from frozen surimi from tropical fish containing cryoprotectants is scarce. The appropriate development of protein film with the satisfactory property involving the improved water barrier property without the impeded mechanical property from surimi should be an alternative promising means to obtain the biodegradable film from marine resources.

Literature Review

1. Edible films and biodegradable films

Edible films and biodegradable films which are able to extend food product shelf-life and preserve food quality have been considered to provide the advantages in the food industry. Films are used in the confectionery, fruits and vegetables, meat, and pharmaceutical industries (Kester and Fennema, 1986; Herald et al, 1995; Krochta and Mulder-Johnston, 1997). Films can prevent the food from interaction with its environment, gains or losses moisture or aroma, taking up oxygen, or contamination with microorganisms (Kester and Fennema, 1986). Furthermore, edible films and biodegradable films can be used to incorporate various food additives, such as flavorings, antimicrobial agents and antioxidant agents, into foods at specific locations. This approach can be used to impart a strong localized functional effect, without elevating excessively the overall concentration of the additive in the food (Kester and Fennema, 1986).

Biopolymers, including protein, polysaccharides, lipids or a combination have been used to produce edible films and biodegradable films (Kester and Fennema, 1986; Gennadios and Weller, 1990; Avena-Bustillos and Krotha, 1993). Protein films are good oxygen- and carbondioxide-barriers properties but show the poor water-vapor barrier properties (Stuchell and Krochta, 1995).

Several approaches can be used to form edible films and biodegradable films (Kester and Fennema, 1986) as follows:

1. **Simple coacervation:** A single hydrocolloids is driven from aquesous suspension or caused to undergo a phase change by evaporation of solvent, addition of a water-miscible nonelectrolyte in which the hydrocolloids is not soluble (e.g., alcohol), addition of an electrolyte to cause salting out or crosslinking, or alteration of pH.

2. **Complex coacervation:** Two solutions of oppositely charged hydrocolloids are combined, causing interaction and precipitation of the polymer complex.

3. **Thermal gelation or precipitation:** A sol-gel transformation can occur by heating of a protein to cause denaturation followed by gelation (e.g., egg albumin) or precipitation, or simple cooling of a warm hydrocolloid suspension.

2. Protein films

Proteins cover a broad range of polymeric compounds that provide structure or biological activity in plants or animals. Various proteins can be used as film-forming materials such as peanut protein (Jangchud and Chinan, 1999), soy protein isolate (Rhim et al., 2002), sunflower protein isolate (Orliac et al., 2002), whey protein (Kim and Ustunol, 2001), egg white protein (Gennadios et al., 1996) and fish myofibrillar protein (Cuq et al., 1995; 1996a; 1996b; 1999; Paschoalick et al., 2003; Shiku et al., 2003).

Protein-based films generally have the superior mechanical and barrier properties to polysaccharide-based films (Cuq et al., 1995). Proteins consisting of about 20 different amino acids have a specific structure which confers a wider variety of functional properties, compared with polysaccharides which are mostly

homopolymers. Furthermore, the chemical treatment to modify functional properties can be performed more easily on protein-based raw materials than on polysaccharide-based raw materials (Osawa and Walsh, 1993). Properties of protein-based films are most likely dependent on the sequential order of the amino acids and protein structure (globular or fibrous).

3. Protein film formation and properties

Protein-based films can be formed in three steps (Figure 1) (Marquie and Guilbert, 2002):

1. Break intermolecular bonds (non-covalent and covalent bonds) that stabilize polymers in their native forms by using chemical or physical rupturing agents (by solubilization or thermal treatment). Polymer chains become mobile.

2. Arrange and orient mobile polymer chains in the desired shape.

3. Allow the formation of new intermolecular bonds and interactions to stabilize the three-dimensional network. The shape obtained in step 2 is maintained by eliminating agents used in step 1 (e.g., solvent removal or cooling).

Based on these three steps, solvent process is based on dispersing and solubilizing the proteins in various solvents and then casting, spraying, or dipping, followed by drying. This process has been extensively studied and applied to produce films from various proteins, particularly from myofibrillar proteins (Cuq et al., 1995).
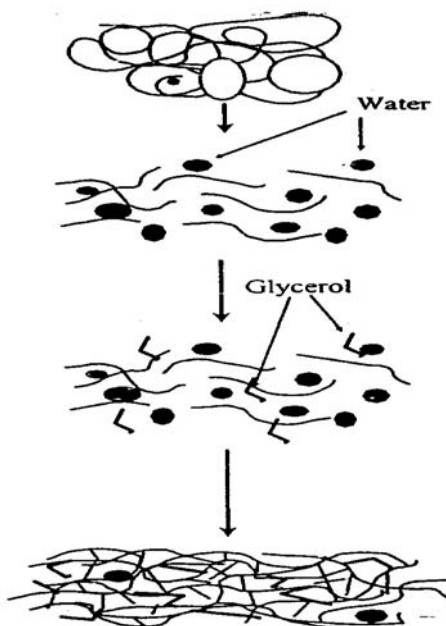
Figure 1: Mechanism of film formation

Source: Adapted from Marquie and Guilbert (2002)

Protein films possess different properties depending upon the sources of protein, protein concentration, extrinsic factors, etc.

1. Barrier properties

Protein films provide the advantage of being excellent oxygen and carbondioxide barriers (Kester and Fennama, 1984; Kester and Fennama, 1989; Gennadios et al., 1993), but their hydrophilic nature makes them rather ineffective moisture barriers (McHugh and Krochta, 1994a; Roy et al., 2000). Protein films have poor moisture resistance due to the inherent hydrophilicity of the proteins and the substantial amounts of hydrophilic plasticizers used to impart film flexibility (Rhim et al., 2000). However, barrier property can be varied with the source of

protein, which can be associated with amino acid composition (Table 1) (Cuq et al, 1995).

Table 1 Water vapor permeability of various protein films.

Film	Water vapor permeability $(x10^{-12}mol.m./m^2.s.Pa)$	Temp (°C)	Thickness (μm)	RH% conditions
Sodium caseinate film	24.7	25	-	100-00
Soy protein film (pH=3)	23.0	25	83	100-50
Corn zein film	6.45	21	200	85-00
Wheat gluten film	5.08	30	50	100-00
Myofibrillar protein film	3.91	25	60	100-00

Source: Adapted from Cuq et al. (1995)

2. Mechanical properties

Generally, the mechanical properties of protein films are poorer than synthetic films (Cuq, 2002; Gennadios et al., 1994). Several factors, including surface charges, hydrophobicities, polymer chain length, etc., may significantly affect the mechanical properties of protein films (Kester and Fennema, 1986). Hydrogen bonds are considered important in contributing to the tensile strength (TS) of protein films (Meier, 1990). Type and level of plasticizer have a dramatic effect on film properties (Shellhammer and Krochta, 1997; Cuq, 2002). Lim et al. (1998) reported that egg white films with higher glycerol contents have greater elongation at break (EAB)

values. Soy protein isolate films containing oleic acid at 10% w/w of soy protein isolate had an EAB of 228% versus an EAB of 70% for the control films. Lipids and fatty acids generally lack the structural integrity of proteins, leading to the reduction of film TS (Gennadios et al., 1998). Myofibrillar based-films had greater TS and lower EAB values when compared with other films (Table 2). The distribution and concentration of inter- and intra-molecular interactions allowed by primary and spatial structures most likely affect the mechanical properties of myofibrillar based-films.

Table 2 Tensile strength and elongation at break of various films.

Film	Tensile strength (MPa)	Elongation at break (%)	Thickness (μm)
Fish myofibrillar protein	17	23	34
Whey protein isolate	14	31	110
Soy protein (pH 9)	3.6	160	83
Wheat gluten (pH 11)	3.3	192	150
Corn zein	3.9	213	67

Source: Adapted from Cuq (2002)

3. Solubility properties

Film solubility is an important property that relates to intended use. High molecular weight proteins are generally insoluble or slightly soluble in water and thus have potential for forming water-resistant films (Cuq, 2002). Low molecular weight protein chains such as monomers and small peptides, formed during the film-forming solution and immobilized in the film net work, could thus constitute the

water-soluble proteinic component of the films (Cuq et al., 1995). Regardless of plasticizer type (glycerol, sorbitol or sucrose), the increase in plasticizer content in the film normally increased the water-soluble dry matter content. In general, hydrophilic plasticizer enhance water solubility (Cuq, 2002; Shiku, 2004). Cuq et al. (1996a) reported that the thickness variation of myofibrillar protein-based films seemed to have no influence on percent solubility in water. Shiku et al. (2004) reported that the film solubility of surimi films was not significantly affected by the quality of surimi.

4. Effect of pH on the properties of protein-based film

Proteins are amphiphilic compounds consisting of polar and nonpolar amino acid residues. Protein solubility is prerequisite of film formation (Cuq, 2002). The pH-dependence of protein solubility is the consequence of its polyionic character. At pH values above or below pI, all of the protein molecules become charged. As a consequence, protein molecules repel each other, and are effectively solvated by water molecules, and are thus more soluble. When the pH is equal to pI (isoelectric point), large dipoles of protein molecules attract themselves through the countercharged domains. There is no electrostatic repulsion between neighboring molecules and they tend to coalesce and precipitate (Sikorski, 2001).

Protein film formation is pH dependent. Gennadios et al. (1996) reported that pH above 12 should be avoided as the film-forming solutions of egg white protein become too viscous, or coagulate during thermal treatment such that the solutions could not be easily cast. The pH of the film-forming solution affects the WVP and mechanical properties of wheat gluten films (Gontard et al., 1992; Gennadios et al., 1993). Gontard et al. (1992) demonstrated that wheat gluten film

properties were highly dependent on film-forming conditions. Both pH and ethanol concentration affect film opacity, solubility and water vapor permeability (WVP). Wheat gluten concentration and pH of the film-forming solution were the most important factors determining film mechanical properties. High wheat gluten concentration induced the formation of a resistant film with high puncture strength. The most resistant film (puncture strength of 4.5 N) was obtained at high wheat gluten concentration (12.5 g / 100 ml) and pH above 5.0. When pH decreased, the effect of wheat gluten concentration on film puncture strength was less pronounced. The saddle surface shape from response surface analysis indicated an optimal pH at about 4.0. Greater film strength could involve a higher number and/or a better localization of bonds among protein chains.

Gennadios et al. (1993) found that wheat gluten films produced at alkaline conditions had greater TS than films produced at acid conditions, but that EAB and WVP were not affected. They also reported that soy protein isolate films prepared from solutions at pH 6-11 had lower WVP and higher TS and EAB than films prepared from pH 1-3 solutions. However, wheat gluten films produced by Gontard et al. (1992) at acid conditions had significantly lower WVP than those produced at alkaline conditions.

Edible films made from soy protein isolate had the varying properties depending on pH used for film preparation (Brandenburg et al., 1993). Alkali-treatment increased percent elongation, but WVP and TS were not affected by alkali-treatment. A minimum pH of 8 was needed when using ammonium hydroxide as alkali source to yield a satisfactory film. The pH above 8 did not provide the improvements in film properties. Avena-Bustillos and Krochta (1993) investigated the

effects of pH adjustment, calcium crosslinkage, and lipid content on the WVP of caseinate-based edible films. Films were dipped in calcium ascorbate buffer for one minute to adjust the pH to 4.6 (isoelectric point) and calcium ion induced the cross-linking of the films. The addition of acetylated monoglyceride resulted in WVP reduction.

Pérez-Gago et al. (1999) reported that the pH of the film-forming solution of whey protein film did not significantly affect film solubility, mechanical properties, and WVP at pH away from the pI. The increase of WVP observed at the pI was likely due to incomplete removal of air bubbles because of increased film-forming solution viscosity. Handa et al. (1999) correlated the mechanical properties of egg white films with the surface concentrations of SH groups in egg white film-forming solutions. Films cast from heated solutions had slightly greater TS than those from unheated solutions, but in most cases, TS of film was not affected by pH. Films prepared from heated solutions had greater EAB than those prepared from unheated solutions only at the most alkaline condition (pH 11.5) used, but film EAB values increased with pH in most cases. Concentration of surface SH groups correlated positively with EAB. It was concluded that the increase in surface SH groups due to heat and alkaline treatments resulted in increased S-S bonding in the egg white films, which rendered the films more stretchable (Handa et al., 1999). Jungchud and Chinnan (1999) showed that peanut protein films plasticized with glycerol also increased their TS when the pH increased.

Myofibrillar proteins are sensitive to pH variations because of their particularly high contents of ionized polar amino acids (about 27-31%) (Orban et al., 1992). Film formation by the solvent process requires dissolution of myofibrillar

proteins by adjusting the pH of the film-forming solution. Cuq et al. (1995) reported that optimal conditions for preparing film-forming solutions based on myofibrillar proteins were at pH of 3 and 2 g protein/100 g solution, where homogeneous film-forming solution with low viscosity was obtained and myofibrillar proteins do not precipitate out of solution and solution can be easily spread into thin layer. Shiku et al. (2003) examined the effect of pH on the preparation of edible films based on fish myofibrillar proteins. Myofibrillar protein-based films were formed at pH ranges of 2-3 and 7-12, whereas films were not formed between pH 4 and 6 because of the poor protein dispersion around the isoelectric point. TS of the films was higher when prepared at the acidic (pH 2, 3) and alkaline (pH 11, 12) conditions, whereas EAB was almost constant irrespective of pH. Nevertheless, pH of film-forming solutions had no effect on WVP of the film.

5. Effect of plasticizers on the properties of protein-based film

Protein films are often quite stiff and brittle due to extensive interactions between protein chains through hydrogen bonding, electrostatic forces, hydrophobic interaction, and/or disulfide cross-linking. Normally, plasticizers including mono-, di-, and oligosaccharides, polyols and lipids are added in order to improve film flexibility by reducing those interactions. (Avena-Bustillos and Krochta, 1993; Shellhammer and Krochta, 1997). Plasticizers reduce internal hydrogen bonding and increase intermolecular spacing, thereby decreasing brittleness and increasing permeability of film materials (McHugh et al., 1994) while decreasing TS (Gennadios et al., 1996; Jangchud and Chinan, 1999). McHugh et al. (1994) found

that the types of plasticizers had the marked effect on the WVP of whey protein edible films.

Glycerol and sorbitol plasticizers act by reducing internal hydrogen bonding in films, thereby increasing film flexibility while increasing WVP. Gennadios et al. (1993) reported that hydrophilicity of film polymers was associated with the sensitivity of film to the humidity. Since plasticizer incorporation affected hydrophilicity of soy protein isolate films, mechanical properties of soy protein isolate films varied with plasticizers and their concentrations (Gennadios et al., 1993). Jangchud and Chinan (1999) reported that glycerin was found to be the most suitable plasticizer to incorporate into peanut protein film when compared with sorbitol, propylene glycol and polyethylene glycol. Lim et al. (1999) found that gelatin films with higher glycerol content exhibited higher equilibrium moisture content, indicating higher hydrophilicity of the films. Absorbed water and glycerol in the film worked synergistically, resulting in the greater flexibility. Banerjee and Chen (1995) reported that using glycerol as a plasticizer in whey protein isolate films resulted in the large moisture content values. Coupland et al. (2000) reported that the equilibrium moisture content of the whey protein isolate films increased linearly as glycerol concentration increased. However, increasing the polyol chain length induced slightly higher surface hydrophobicity but poor mechanical properties of the films (Viroben et al., 2000). Chick and Ustunol (1998) showed that casein-based film plasticizered with glycerol had higher WVP values than films plasticizered with sorbitol when the same amounts of plasticizer were used.

Gennadios et al. (1996) reported that egg albumen films with sorbitol as plasticizer had lowest WVP when compared with those containing glycerin and

polyethylene glycol. Cuq et al. (1997) reported that plasticization of myofibrillar protein-based films with glycerol, sorbitol or sucrose induced large decreases in film strength and elasticity but increases in deformation and WVP. Tanaka et al. (2001) found that WVP of edible films prepared from fish water-soluble proteins decreased with increasing concentration of glycerol. However, the increased ratio of glycerol to polyethylene glycol reduced the water vapor barrier properties of films.

The effects of glycerol and polyethylene glycol (PEG) 400 plasticizers on the TS of sodium caseinate films and "cross-over" phenomenon were reported by Siew et al. (1999). Films plasticized with glycerol had higher TS at low plasticizer concentration, but showed lower TS at higher plasticizer concentration than film plasticized with PEG. PEG system had a more homogeneous bonding distribution, while glycerol, a smaller plasticizer molecule, can access the hydrophilic sites on the caseinate chain more easily than large plasticizer molecules. Glycerol can be easily inserted between the protein strands through hydrogen bonding, thus reducing intermolecular protein interactions, increasing intermolecular spacing, and lowering the mechanical strength of casein films. Galietta et al. (1998) investigated the plasticizing effect of glycerol on mechanical properties of whey protein isolate films. Increased plasticizer content resulted in the increased film solubility in water and decreased the force at break and Young's modulus values.

6. Effect of lipids on the properties of protein-based film

Most single hydrophilic films (protein based-films) have good mechanical properties and are excellent gas, aroma and lipids barriers but poor moisture barriers. Serveral investigators have attempted to improve the moisture barrier properties of hydrophilic films by incorporation of lipids through emulsion (Kamper and Fennema, 1984; Kester and Fennema, 1989; Shellhammer and Krochta, 1997). The barrier efficiency of resulting composite films strongly depends on polarity of films components and distribution of lipid material in the film matrix (Kamper and Fennema, 1984; Debeaufort et al., 1993). The water vapor barrier ability of protein films can be improved using hydrophobic lipid materials (Gennadios et al., 1994; Krochta and De Mulder-Johnston, 1997). Kamper and Fennema (1984) reported that WVP of emulsion films varied greatly with film composition and orientation of molecules. McHugh and Krochta (1994a) examined the effect of incorporating various lipids into whey-protein-lipid-emulsion edible films. Among all lipids including acetylated monoglycerides, waxes, fatty alcohols and fatty acids, the beewax emulsion film had the lowest WVP. Increasing lipid chain length generally decreased the WVP of whey-protein-lipid-emulsion edible films (McHugh and Krochta, 1994b). Sherwin et al. (1998) reported that increasing fatty acid chain length increased the particle size in the emulsion films. Lipid type and amount affected the WVP of dispersed-lipid films prepared with whey protein isolate (WPI) and glycerol (GLY) (WPI:Gly ratio of 15:1) (Shellhammer and Krochta, 1997). For all emulsion films containing candelilla wax (CanW), carnauba wax (CarW), beeswax (BW), and a hard anhydrous milkfat fraction (HAMFF), WVP decreased with increasing lipid concentration.

pH of film-forming solution also had the influence on the emulsion-type films (Pérez-Gago and Krochta, 1999). Pérez-Gago and Krochta (1999) examined the role of emulsion stability as affected by pH on the final morphology and WVP of WPI-lipid emulsion films. The films were cast from aqueous solutions of heat-denatured WPI (5% w/w), beeswax (liquid phase), and glycerol. In general, there was some phase separation and no significant difference among WVP of emulsion films prepared at pH 6, 7, or 8. Inhibited lipid particle coalescence at the pI (pH 4-5) resulted in the increased WVP. Emulsion viscosity increased at the pI and a weak gel was formed due to protein-protein aggregation. This lowered lipid mobility, thus prohibiting any phase separation and resulting in higher film WVP. Debeaufort et al. (1993) found that the decrease of lipid particle size correlates well with the reduction of WVP.

Composite protein-lipids films had lower WVP values than control protein films from caseinates (Avena-Bustillos and Krochta, 1993). Park et al. (1994) prepared laminated methyl cellulose/corn zein-fatty acid films by casting corn zein-fatty acid solutions onto methyl cellulose films. WVP decreased as chain length and concentration of fatty acid increased. The TS of laminated edible film containing palmitic acid decreased as palmitic acid increased. The TS of film containing stearic-palmitic acid blends showed similar trends but there were no significant differences among blends. The TS of the film containing lauric acid was maximal at 30% lauric acid concentration. The EAB values for films containing fatty acids varied inversely with TS.

Properties of gluten-based composite films depended on the lipid characteristics and on the interactions between the lipid and the protein structural matrix (Gontard et al., 1994). Among various lipids, beeswax that a solid and highly hydrophobic lipid was the most effective lipid for improving moisture barrier properties of films; but these films were opaque, weak and disintegrated easily in water. WVP of laminate whey protein-lipid films decreased by 70 times (Anker et al., 2002). The WVP of emulsion whey protein-lipid films was half the value of the pure whey protein films and was not affected by changes in lipid concentrations, whereas an increased homogenization led to a slight reduction of the WVP (Anker et al., 2002).

7. Protein-based film from different sources

7.1 Wheat gluten film

Wheat gluten is defined as the water-insoluble protein of wheat flour. Wheat gluten contains the prolamine and glutelin fractions of wheat flour protein, typically referred to as gliadin and glutenin, respectively (Krochta, 2002). Gliadin is soluble in 70% ethanol, but glutenin is not. Both gliadin and glutenin fractions of wheat gluten contain intramolecular disulfide bonds. Intermolecular disulfide bonds, which link individual glutenin protein chains, result in the larger polymers with high molecular weight. The extensive intermolecular interactions in wheat gluten result in quite brittle films with poor water-vapor barrier properties (Gennadios and Weller, 1990). Herald et al. (1995) reported that films prepared from spray-dried (SD) and flash-dried (FD) wheat gluten had differences in properties. Films from wheat gluten are comparable to plastic wrap for most properties except WVP. SD wheat gluten

films exhibited a higher tensile strength (TS) than did the FD wheat gluten films and plastic wrap (Table 3).

Table 3 Comparison of specific mechanical and barrier properties of plastic wrap and spray-dried (SD) and flash-dried (FD) wheat gluten films.

Parameters	FD film	SD film	Plastic Wrap
Film thickness (μm)	167	189	30
WVP (gm/m^{2}sPa)	7.7×10^{-9}	7.1×10^{-9}	3.09×10^{-13}
Tensile strength (MPa)	1.19	2.12	0.0643

Source: Adapted from Herald et al. (1995)

7.2 Casein films

Casein, which comprises 80% of milk protein, precipitates when skim milk is acidified to the isoelectric pH, approximately of 4.6 (McHugh and Krochta, 1994c). Film formation of aqueous casein solution without heat treatment was due to their random-coil nature. Interactions in the film matrix likely include hydrophobic, ionic and hydrogen bonding (Avena-Bustillos and Krochta, 1993). Tomasula et al. (1998) reported that a minimal glycerol concentration of 20% was necessary to prepare casein films suitable for mechanical testing. Casein films cast from solutions with total solids contents of 2 or 4% were brittle and difficult to peel from the casting surface (Tomasula et al., 1998).

7.3 Whey protein films

Whey protein comprising 20% of milk protein is the protein that remains soluble after casein is precipitated at pH 4.6. Whey protein consists of several proteins, which are globular and heat labile in nature (McHugh et al.,1994). Because of the globular nature of whey proteins, the formation of films requires heat denaturation to open the globular structure, break existing disulfide bonds, and form new intermolecular disulfide and hydrophobic interactions (McHugh et al., 1994). McHugh et al. (1994) suggested that the best film formation conditions were 10% (w/w) protein solutions with neutral pH and heated for 30 min at 90ºC.

7.4 Corn zein film

The zein, which is a prolamine, is soluble in 70% ethanol. In terms of the amino acid composition, zein has a high content of nonpolar hydrophobic amino acids such as leucine, alanine and proline. Zein also contains a high level of glutamic acid (about 20-22%), which exists mostly as glutamine. Glutamine contributes to the insolubility of zein in water (Gennadios and Weller, 1990). Therefore, zein films are generally cast from alcohol solutions (Gennadios et al, 1993). Tensile strength (TS) of zein films was similar to that of wheat gluten films and had WVP values lower than or similar to those of other protein films (Guilbert, 1986). Parris et al. (1997) proposed a new method for isolating zein from rinsed dry milled corn by extraction with 70% (v/v) ethanol. Films prepared from isolated zein, containing 1% starch, had lower WVP values and were more water resistant than those prepared from commercial zein. However, water resistance decreased with increasing starch content.

7.5 Myofibrillar protein film

Myofibrillar proteins are found in meats and fish, representing the main component of muscles (approximately 40-60%) (Suzuki, 1981). These proteins represent significant sources of proteins rich in essential amino acids (Cuq, 2002). High molecular weight proteins e.g., myosin and fibrous proteins e.g., myosin and F-actin are generally able to form films with good mechanical properties (Cuq, 1998).

Myofibrillar proteins can be used for film-forming application after purification and concentration from meat or fish. This involves successive washing treatments to remove undesirable compounds such as blood components, sarcoplasmic proteins (myoglobin), extracellular proteins (collagen) and lipids (Cuq, 1995). The process is known as surimi process. However, Paschoalick et al. (2003) suggested that the presence of sarcoplasmic proteins did not affect the quality of functional properties of films based on muscle proteins of Nile Tilapia. Shiku et al. (2004) reported that transparent and flexible protein films were successfully made from frozen Alaska pollack surimi. The quality of surimi was reported to affect the resulting film. Slight protein denaturation caused the decrease in elongation at break (EAB) of the films and complete protein denaturation gave rise to the reduction of TS and EAB. Myofibrillar protein-based films have interesting functional properties such as mechanical or water vapor barrier properties (Cuq et al., 1995; 1996a; 1996b; 1999; Paschoalick et al., 2003; Shiku et al., 2003).

7.6 Sarcoplasmic protein films

Sarcoplasmic protein can be used for film preparation (Iwata et al., 2000; Tanaka et al., 2001). Transparent sarcoplasmic protein films were reported to exhibit better flexibility and lower WVP, compared with most of the other protein films (Iwata et al., 2000). The functional properties of sarcoplasmic protein-based films are sensitive to heating because their globular proteins must be thermally denatured to form a continuous matrix (Iwata et al., 2000).

7.7 Gelatin film

Thermally reversible gels are formed by heating aqueous solutions of gelatin, followed by cross-linked between amino and carboxyl components of amino acid residue side groups (Glicksman, 1982). Film produced by drying gelation gels are poor moisture barriers. Guibert (1986) found that cross-linking/ denaturing gelatin films with calcium ions had no effect on water-barrier properties. Arvanitoyannis et al. (1997) elaborated films with blends of equal parts of gelatin and soluble starch, using sorbitol as plasticizer. The increment of sorbitol from 15 to 30% increased the WVP values of the film.

8. Improvement of protein films properties

Films can be strengthened by cross-linking agents that chemically modify proteins during preparation of film-forming solutions. Cross-linking agents are natural or synthetic molecules containing at least two reactive groups that are able to form covalent inter- and/or intra-molecular links between protein chains. These agents, when used to prepare protein-based films, strengthen the material through formation of new covalent bonds, while reducing film elasticity and solubility in

water (Gennadios and Weller, 1992). Aldehydes such as formaldehyde, glutaraldehyde or glyoxal have been used to cross-link cottonseed protein (Marquie, 2001), wheat gluten (Hermáñdez-Muñoz et al., 2004) and soy protein (Rhim et al, 2000). Formaldehyde, the simplest of cross-linking agents, has the broadest reaction specificity. In addition to amino group of lysine, it reacts with the side chains of cysteine, tyrosine, histidine, tryptophan and arginine (Tae, 1983). Although formaldehyde contains a single functional group, it can react bifunctionally and therefore effectively crosslink the proteins. Glutaraldehyde is more specific than formaldehyde. It can react with lysine, cysteine, histidine and tyrosine. Protein cross-linking by glyoxal involves the reaction with lysine and arginine side chain groups (Marquie and Guilbert, 2002) (Figure 2).
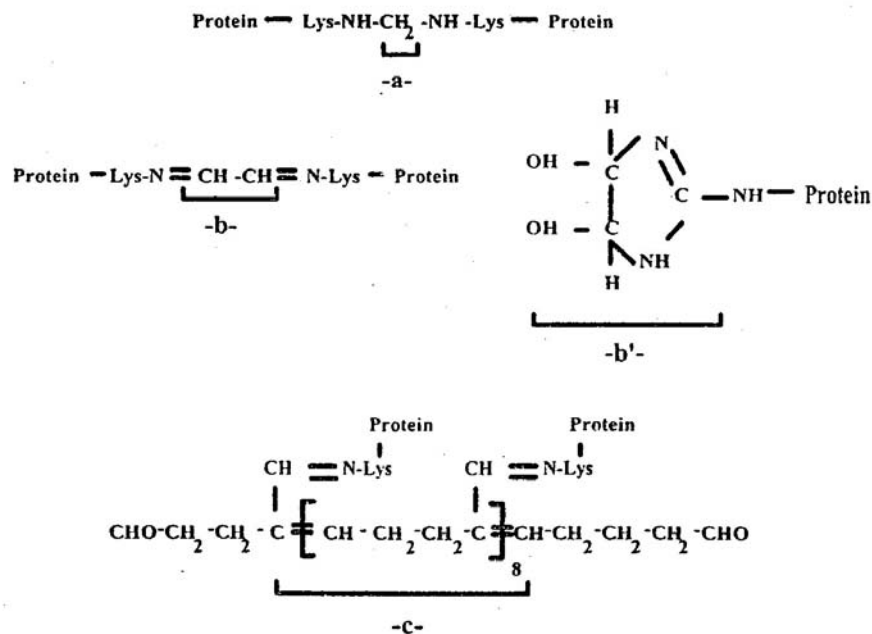

Figure 2 Mechanism of protein cross-linking by formaldehyde, glyoxal or

glutaraldehyde; (a) methylene cross-links formed with formaldehyde reaction;
(b) cross-links and (b') cyclic compounds formed with glyoxal; and (c) polyglutaraldehyde cross-links.

Source: Marquie and Guilbert (2002)

Chemical modification has been reported to affect the protein film property. With formaldehyde modification, a slight reduction in WVP in gluten protein based films (Micard et al., 2000) and in films based on soy protein (Ghorpade et al., 1995) were found. The action of formaldehyde was more pronounced in lowering the WVP than that of glyoxal in gelatin based films (de Carvalho and Grosso, 2004).

The variation in mechanical properties of the formaldehyde modified films depended on the ability of proteins to form chemical and physical bonds simultaneously, and the final effect of these bonds on the orientation of the protein (Fakirov et al., 1996). Wheat gluten based films treated with formaldehyde showed an increase in TS but a decrease in EAB as compared to the untreated control films (Micard et al., 2000). Formaldehyde added in the film-forming solution did not alter the mechanical behaviour of pea protein isolate film, whereas treatment by immersion of the protein films in an ethanol-formaldehyde mixture markedly increased both their TS and hydrophobic character (Gueguen et al., 1998).

Formaldehyde can be directly added to soy protein isolate film-forming solutions or it can be applied by immersing dried soy protein isolate films into formaldehyde solutions (Rhim et al., 2000). Treatment with formaldehyde resulted in the increases in TS and puncture strength by two folds, but reduced WVP (by about 6%) and the water solubility (by about 42%) of soy protein isolate films.

The oxygen permeability of the formaldehyde-treated soy protein isolate films increased slightly (Ghorpade et al., 1995). Galietta et al. (1998) reported that formaldehyde, as a cross-linking agent, enhanced the mechanical properties and insolubility in water of whey protein isolate films. Formaldehyde and glutaraldehyde addition caused a significant increase in the TS of peanut protein films compared to the control films (Liu et al., 2004). The WVP and oxygen permeability of the films decreased after aldehyde treatment. The ability of formaldehyde and glutaraldehyde to promote covalent intermolecular cross-linking of peanut protein film was therefore effective to increase the mechanical and barrier properties of the films. Bigi et al. (2001) found that the degree of cross-linking of gelatin based film induced by glutaraldehyde related with the increases in TS and decreases in elasticity of film. Audic and Chaufer (2005) showed that chemical cross-linking between formaldehyde and free amino acid groups of sodium caseinate caused the increase in water resistance of triethanolamine (TEA) plasticized films.

Parris and Coffin (1997) reported a reduction in WVP when polymeric dialdehyde starch was added (at 20% w/w of zein) to zein films without the addition of plasticizers. However, addition of polymeric dialdehyde starch did not affect WVP when glycerol or polypropylene glycol were added to the films. Gennadios et al. (1998) reported that TS and EAB increased significantly with increasing amounts of dialdehyde starch added to egg white films (optimal dialdehyde starch level ≈ 5% w/w of egg white), suggesting the formation of covalent cross-links between egg white proteins and dialdehyde starch. Soy protein isolate films containing dialdehyde starch at 5 or 10% w/w of soy protein isolate had the increase in TS by about 20%, compared to the control soy protein isolate films (Rhim, 1998). Soy protein isolate

films containing dialdehyde starch (10% w/w of soy protein isolate) were insoluble in water and other solvents (i.e., 0.01 N hydrochloric acid, 0.01N sodium hydroxide, 4 M urea, and 0.2 M 2-mercaptoethanol) (Rhim et al., 2000).

Additionally, the mechanical properties of protein based film can be improved by transglutaminase (TGase, protein-glutamine γ-glutamyltransferase, EC 2.3.3.13). TGase is an enzyme that catalyzes acyl-transfer reaction between γ-carboxyamine groups of glutamine residues in protein, peptides, and various primary amines. When the ε-amino groups of lysine acts as acyl acceptor, it results in polymerization and inter- or intra-molecular cross-linking of proteins such as the formation of ε-(γ-glutaminyl) lysine (Nielsen, 1995; Lim et al., 2002) (Figure 3). In the absence of primary amines, water may act as the acyl acceptor, resulting in deamination of γ-carboxyamine groups of glutamine to form glutamic acid (Ashie and Lanier, 2000). Transglutaminase catalyzes the formation of intermolecular and intramolecular covalent bonds between and within protein chains, resulting in a broad size distribution of protein molecules (Aboumahmoud and Savello, 1990).
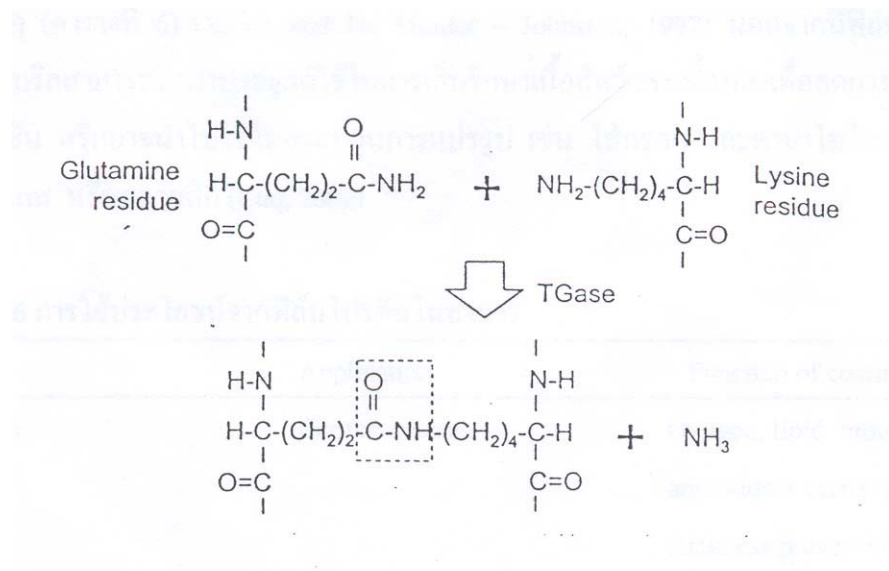
Figure 3 Transglutaminase-catalyzed cross-linking reaction in proteins.

Source: Lim et al. (2002)

Transglutaminase-catalyzed reactions to cross-link soy bean 11S globulin and whey protein isolate gave biopolymers with improved functionality (Yildirim et al., 1996). Cross-linking of proteins provided biopolymers with improved heat stability. Since heat treatment generally leads to loss of solubility, the heat stable nature of such biopolymers enables them to stay in solution at higher temperatures (Yildirim and Hettarachchy, 1997). Yildirim and Hettarachchy (1998) compared the properties of transglutaminase cross-linked whey protein isolate films, soy bean 11S and a mixture of these two protein (1:1, w/w). TS values of film added with 0.2 units TGase/g proteins were two-fold greater than that of the control. Transglutaminase cross-linked films was lower in solubility than the control films at pH 3, 4, 6, or 8. Mahmoud and Savello (1993) reported that the increase in the degree of cross-linking resulting from the action of TGase on whey protein produced less soluble films in various conditions of pH and heat treatment, but maintaining the enzymatic

digestibility characteristics. Mariniello et al. (2003) reported that edible pectin-soy flour films in presence of transglutaminase had the increased strength but reduced flexibility.

9. Stability of protein-based films during storage

Natural polymers are much less stable than most synthetic materials. The use of biopolymers as the sole packing raw material is being delayed because the main functional properties highly depend on external conditions such as temperature and relative humidity (RH). Cuq et al. (1996) reported that solubility in water, WVP and mechanical properties of myofibrillar protein based-films remained constant for 8 weeks at 20ºC and 58%RH. However, the film turned to be slightly yellowish. Somanathan et al. (1992) reported that triethanolamine-treated casein films became brown in color and were considerably less resistant after 1 year of storage at 25ºC and 65%RH. Glycerol could migrate slowly from the film bulk to the surface of gluten-based films during storage at 25ºC and 50%RH, even when glycerol was initially well dispersed in the film-forming solution (Park et al., 1994). Soy protein isolate film underwent different changes during storage at 25ºC depending on the water content in the container (Park and Hettiarachchy, 2000). The degree of degradation of soy protein isolate films was greater at 25ºC than at 15ºC. The degradability of the soy protein isolate films during storage appeared to be more sensitive to moisture than to temperature.

10. Applications of protein based-films

Protein based-films have been used to protect and to improve the shelf life of food products (Table 4) (Kroch and De Mulder–Johnston, 1997). Protein based-films have been applied for coating nuts or adding in bakery products (Gennadios and Weller, 1990). Whey protein film is used for coating fresh vegetables as well as the addition in chocolate (McHugh and Krochta, 1994c).

Table 4 Applications of protein based-films in foods.

Materials	Application	Function of coating
Corn zein	Confectionaries	Oxygen, lipid, moisture barrier; antioxidant carrier and stickness prevention
	Tomatoes	Moisture and oxygen barrier
Whey protein	Peanuts	Oxygen barrier
	Frozen salmon	Antioxidant carrier
Wheat gluten	Nuts	Salt binding
Casein	Peanut	Oxygen barrier

	Frozen salmon	Antioxidant carrier

Source: Adapted from Kroch and De Mulder – Johnston (1997)

The use of films prepared from spray-dried wheat gluten as coating material resulted in maintaining quality of Grade A quality shell eggs for 30 days at room temperature (Herald et al., 1995). Avena-Bustillos et al. (1993) studied the potential of caseinate-acetylated-monoglyceride films on peeled carrots and found that water vapor resistance of samples increased, compared with the controls. Caseinate-acetylated monoglyceride emulsions were applied on whole fruit apples and celery sticks (Avena-Bustillos et al., 1997). The coating improved the water vapor resistance of celery sticks with an optimal formulation of 1.5% calcium caseinate and 1.5% acetylated monoglyceride. However, the coating did not affect water vapor resistance, respiration rate, or ethylene production of whole apples. It was also reported that water vapor resistance was not affected by the type of caseinate, calcium cross-linking, or addition of potassium sorbate (Avena-Bustillos et al., 1997).

Stuchell and Krochta (1995) showed that frozen king salmon coated with an edible whey protein-lipid solution had the decrease in moisture loss by 42-65% during three weeks of storage at -23ºC. Xu et al. (2001) reported that the shelf life of kiwifruit coated with edible film comprising soybean protein isolate, stearic acid and pullulan extended to about 3 times, compared with the control.

Soy protein films was used for coating foods to reduce oil uptake during deep-fat frying (Rayner et al., 2000). Coated and fried discs of doughnut mix had a notably reduced fat content (by about 55%) compared to non-coated samples. Also, a preference evaluation by an untrained sensory panel indicated no significant

difference between coated and non-coated French fries (Rayner et al., 2000). Wrapping bread samples in the casemate films reduced hardness during 6-h storage at ambient temperature effectively, when compared with unwrapped controls (Schou et al., 2005).

Myofibrillar protein-based films can be used for protecting fish or meat pieces from oxidation or dehydration during storage. For processed meat or fish products e.g., sausages and kamaboko, protein film can be the alternative packaging to replace currently used cellulose coatings or plastic films (Cuq, 2002).