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PROPERTIES OF WHEY PROTEIN/ LIPID EMULSION EDIBLE FILMS

By

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ABSTRACT

PROPERTIES OF WHEY PROTEIN/ LIPID EMULSION EDIBLE FILMS

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Methodologies were developed to produce edible films from whey protein isolate (WPI) and concentrate (WPC), and film-forming procedure was optimized. Lipids, butter fat (BF) and candelilla wax (CW), were added into film-forming solutions to produce whey protein/lipid emulsion edible films. Significant reduction in water vapor and oxygen permeabilities of the films could be achieved upon addition of BF and CW. Mechanical properties were also influenced by the lipid type. Microstructures of the films accounted for the differences in their barrier and mechanical properties. Studies with bond-dissociating agents indicated that disulfide and hydrogen bonds, cooperatively, were the primary forces involved in the formation and stability of whey protein/lipid emulsion films. Contribution of hydrophobic interactions was secondary.

Thermal properties of the films were studied using differential scanning calorimetry, and the results were used to optimize heat-sealing conditions for the films. Electron spectroscopy for chemical analysis (ESCA) was used to study the nature of the interfacial interaction of sealed films. All films were heat sealable and showed good seal strengths while the plasticizer type influenced optimum heat-sealing temperatures of the films, 130°C for sorbitol-plasticized WPI films and 110°C for glycerol-plasticized WPI films. ESCA spectra showed that the main interactions responsible for the heat-sealed

joint of whey protein-based edible films were hydrogen bonds and covalent bonds involving C-O-H and N-C components.

Finally, solubility in water, moisture contents, moisture sorption isotherms and sensory attributes (using a trained sensory panel) of the films were determined. Suitability of WPI-based pouches in packaging of powder cocoa mix was investigated. Solubility was influenced primarily by the plasticizer in the films, and the higher the plasticizer content, the greater was the solubility of the films in water. Moisture contents of the films showed a strong relationship with moisture sorption isotherm properties of the films. Lower moisture content of the films resulted in lower equilibrium moisture contents at all a_w levels. Sensory evaluation of the films revealed that no distinctive odor existed in WPI films. All films tested showed slight sweetness and adhesiveness. Films with lipids were scored as being opaque while films without lipids were scored to be clear. Whey protein/lipid emulsion edible films may be suitable for packaging of powder cocoa mix and should be suitable for packaging of non-hygroscopic foods.

To my parents

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ABBREVIATIONS

ANS	1-anilino-8-naphthalene sulfonic acid
ASTM	American Society for Testing and Materials
BF	Butter fat
CFR	Code of Federal Regulation
CW	Candelilla wax
DTNB	5,5′-dithiobis (2-nitrobenzoic acid)
DSC	Differential scanning calorimetry
%E	Percent elongation
EMC	Equilibrium moisture content
ESCA	Electron spectroscopy for chemical analysis
FDA	Food and Drug Administration
G	Glycerol
GAB	Guggenheim-Anderson-de Boer
GRAS	Generally-recognized-as-safe
HPMC	Hydroxypropyl methylcellulose
IMC	Initial moisture content
2-ME	2-mercaptoethanol
MSI	Moisture sorption isotherm
$NTSB^{2-}$	Disodium 2-nitro-5-thiosulfobenzoate
NTB^{2-}	2-nitro-5-thiobenzoate anion
OP	Oxygen permeability
RFI	Relative fluorescence intensity
RH	Relative humidity
S	Sorbitol
S_o	Hydrophobicity value
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscopy

T_d	Degradation temperature
T_g	Glass transition temperature
T_m	Melting temperature
T_o	Onset transition temperature
T_p	Peak transition temperature
TGA	Thermogravimetric analysis
TS	Tensile strength
WPC	Whey protein concentrate
WPI	Whey protein isolate
WVP	Water vapor permeability

INTRODUCTION

Edible films such as wax coatings, sugar and chocolate covers, and sausage casings, have been used in food applications for years (Guilbert, 1986). However, interest in edible films and biodegradable polymers has been renewed due to concerns about the environment, a need to reduce the quantity of disposable packaging, and demand by the consumer for higher quality food products.

Edible films can function as secondary packaging materials to enhance food quality and reduce the amount of traditional packaging needed. For example, edible films can serve to enhance food quality by acting as moisture and gas barriers, thus, providing protection to a food product after the primary packaging is opened. Edible films are not meant to replace synthetic packaging materials; instead, they provide the potential as food packagings where traditional synthetic or biodegradable plastics cannot function. For instance, edible films can be used as convenient soluble pouches containing single-servings for products such as instant noodles and soup/seasoning combination. In the food industry, they can be used as ingredient delivery systems for delivering pre-measured ingredients during processing. Edible films also can provide the food processors with a variety of new opportunities for product development and processing.

Research on milk protein-based edible films has been reviewed by McHugh and Krochta (1994a). Film formation from nonfat dry milk (NFDM) was interfered due to lactose crystallization, and use of total milk proteins as film-forming materials was only possible after removal of lactose from NFDM (Maynes and Krochta, 1994). Earlier studies with whey protein- and casein-based edible films involved enzymatic crosslinking

of proteins using transglutaminase (Motoki et al., 1987; Mahmoud and Savello, 1992, 1993). Ho (1992) reported on water vapor permeability (WVP) of caseinate films produced without crosslinking enzymes. He also reported on the effect of incorporating lipids on the WVP of these films. McHugh et al. (1994) produced films from whey protein isolate by applying heat and monitoring pH and protein concentration of the film-forming solutions without the use of crosslinking enzymes. Typically whey protein-based films are not good moisture barriers due to the hydrophilic nature of proteins. McHugh and Krochta (1994b) suggested incorporation of lipids into whey protein film-forming solutions since lipids are the most effective edible barriers to moisture transfer. Until now much of the research on milk protein-based edible films have focused on their development and testing for their barrier and mechanical properties. There is very little or no other information available on other properties of these films.

I hypothesize that it is possible to make whey protein-based edible films with improved moisture barrier properties without significantly altering other properties by producing whey protein/lipid emulsion films and these films will be suitable for food applications. The following are the specific objectives of this research:

1. Develop whey protein/lipid emulsion edible films and determine their microstructures, barrier (moisture and oxygen) and mechanical (tensile strength and elongation) properties.
2. Study the nature of interactions involved in the formation and stability of the films.
3. Investigate thermal properties, heat sealability, and sealing properties of the films.
4. Demonstrate suitability of their application in foods as packaging materials.

CHAPTER 1

LITERATURE REVIEW

1.1 EDIBLE FILM

1.1.1 Definition and historical background

Edible film can be defined as a thin and continuous layer of edible materials that can provide a barrier to mass transfer, like moisture, oxygen, lipids and solutes, and/or act as a carrier for food ingredients and additives. Edible films differ from edible coatings in that they are pre-formed and freestanding sheets (Krochta, 1992; Chen, 1995). In ancient China, wax has been used on citrus fruit to delay dehydration and fat coatings have been applied to meats to prevent shrinkage since the 12th century. Yuba, the first freestanding edible film appeared in the 15th century Japan, was obtained from boiled soymilk and was used on food products for preservation and improvement of appearance (Guilbert and Biquet, 1996).

It was in the last few decades that edible films received scientific attention and validation for their potential as food packaging materials. Over the last 40 years, a great number of scientific articles and patents have been published on the characterization and application of edible films, and myriad resources were investigated as edible film-forming materials. Some of these edible films are now available in the commercial market.

1.1.2 Formation of edible films

1.1.2.1 Components of edible films

Formation of films requires use of at least one component capable of forming a structural matrix with enough cohesive strength (Banker, 1966). Hydrocolloids, polysaccharides and proteins, meet this requirement and offer good mechanical strength compared to that of lipids. However, hydrocolloid films are poor moisture barriers and lipids are often combined with them to improve moisture barrier properties by increasing hydrophobicity of the films (Krochta, 1997a; Debeaufort et al., 1998).

The polysaccharides used to form edible films are alginate, dextrin, starch, pectin, carrageenan, chitosan, gum arabic and cellulose derivatives. Proteins used in edible films include wheat gluten, collagen, gelatin, corn, soy, peanut, and milk proteins (Kester and Fennema, 1986). Lipids used in edible films are typically waxes (beeswax, candelilla wax, carnauba wax), surfactants (glycerol monostearate, acetate glycerol monostearate, citrate glycerol monostearate and sorbitol monostearate), and fatty acids (lauric acid, palmitic acid and stearic acid) (Donhowe and Fennema, 1993; Park et al., 1994b; Debeaufort and Voilley, 1995).

Like synthetic films, edible films also often require use of plasticizers to enhance film pliability. Protein-based films in particular by themselves form very brittle films, however the brittleness can be decreased with the aid of a plasticizer. Plasticizers reduce the level of intermolecular interactions in polymer chains and enhance pliability of films. Common food-grade plasticizers include sorbitol, mannitol, sucrose, glycerol, propylene glycerol, polyethylene glycol, triethylene glycol, fatty acid, and monoglycerides (Reiners, 1973; Krochta, 1997b).

A solvent system is required to produce a hydrocolloid or an emulsion film. The solvent allows solubilization and uniform spreading of high molecular weight polymer to form a thin layer film. Water and ethanol are the two typical solvents used for edible film formation (Kester and Fennema, 1986). There are also a number of additives that can be used in the film-forming solutions to influence properties of edible films. These include crosslinkers, various nutrients, flavoring and coloring agents, antioxidants, and antimicrobials (Donhowe and Fennema, 1994; Krochta, 1997b).

1.1.2.2 Manufacture of edible films

Solvent casting is the common process to form hydrocolloid edible films. A film-forming material is dispersed in an aqueous solution, generally water, ethanol, or a combination of both. The film-forming solution is distributed by spreading or pouring it in a thin layer, then allowing it to dry for solvent removal. Dried film is detached from the support and the freestanding film is complete. In the industry, solvent casting has been adopted commercially to manufacture hydroxypropyl methylcellulose (HPMC) edible films (Donhowe and Fennema, 1994; Krochta, 1997b). There are several types of surfaces that can be used for edible film casting, such as glass, teflon (polyetrafluorethylene), polystyrene, plexiglass (polymethacrylate), polyethylene (PE), and polyvinyl chloride (PVC) (Gennadios et al., 1993b; McHugh et al., 1994; Maynes and Krochta, 1994).

Extrusion is another technique to obtain self-supporting films. In the extrusion process, a material is compacted and melted in the heated machine barrel and forced through a die to be shaped as finished products (Koyich, 1992). Extrusion has been applied to produce sausage casings from collagen. Several patents can be found referring

to the production of collagen casing films by extrusion (Lieberman, 1964, 1965 & 1967; Fagan, 1970; Miller, 1972).

1.1.2.3 Forces involved in the formation and stability of protein-based edible films

In general, the protein network formation is resultant of protein-protein and protein-solvent interactions, and a balance between attractive and repulsive forces between polypeptide chains. Hydrophobic interaction (enhanced at high temperature), hydrogen bonding (enhanced by cooling), and disulfide cross-links are known to be the attractive forces in the protein network formation (Cheftel et al., 1985). According to Farnum et al. (1976), the film structure is a protein matrix formed by heat-catalyzed protein-protein interactions with disulfide, hydrogen, and hydrophobic bonds.

Disulfide bonds are oxidized forms of sulfhydryls, formed from free sulfhydryl groups of two cysteine molecules in proteins. These bonds are linked together to form polypeptide chains which contribute to a protein's tertiary structure and produce a film. A heating process is important in the formation of protein film network since it alters the three-dimensional structure of proteins, and reveals free sulfhydryl groups and hydrophobic side chains. For example, heating will expose free sulfhydryl groups in β-lactoglobulin, then sulfhydryl/ disulfide interchange reaction may occur (Gennadios et al., 1994). Hydrolysis with acid or alkali is another factor for denaturation of proteins. An alkaline condition aids film formation because disulfide bonds are cleaved and reduced to free sulfhydryl when dispersed in alkaline condition. Disulfide bonds are reformed upon drying of film solutions.

Hydrogen bond is the electrostatic interaction force between polar molecules. The H atom is shared between a proton donor group (acid) and a proton acceptor group (base), for instance -OH or -NH, and $\diagdown\!\!\diagup$C=O, respectively (Howell, 1991). These intermolecular association results in brittle films. Thus, addition of plasticizers is necessary to disrupt some of these associations and decrease rigidity of the film structure.

Hydrophobic interactions can be defined as the attractive force between nonpolar molecules or nonpolar groups of molecules which induce association of these molecules in an aqueous environment (Stenesh, 1989). The majority of the hydrophobic groups of the native protein exist as buried inside of the molecule, therefore only a small amount of hydrophobic interactions occur when protein is not heated. When heated, the hydrophobic side chains may be exposed, and the hydrophobic interaction can occur. Upon drying, exposed hydrophobic residues come closer due to the evaporation of the solvent and form the intermolecular hydrophobic interactions, which contribute to the formation of protein network (Cheftel et al., 1985).

Forces involved in the formation and stability of protein-based edible films will depend on the protein used and its amino acid composition. According to Gennadios et al. (1994), the film-forming ability of corn zein is primarily through hydrophobic interactions and hydrogen bonding in the protein network. Contribution of disulfide bonds is secondary due to low content of cystine in zein. Rangavajhyala et al. (1997) studied solubilities of soy protein-based films in 2-mercaptoethanol (2-ME; a disulfide bond-dissociating agent) and urea (a hydrogen bond-dissociating agent and also a weak hydrophobic bond-dissociating agent). They concluded that disulfide and hydrogen bonds play an important role in the formation of soy protein-based film matrix. Roy et al. (1999)

investigated molecular properties of wheat gluten-based films using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Reportedly, cross-linking through disulfide bonds was responsible for the polymerization of film-forming solutions. They also suggested that similar film-forming mechanism could be involved for other sulfhydryl groups containing proteins such as soy and whey proteins.

1.2 PROPERTIES OF PROTEIN/ LIPID EMULSION EDIBLE FILMS

Although protein-based edible films show good oxygen barrier properties compared to synthetic films and posses sufficient mechanical strength to be utilized as packaging materials, they are poor water vapor barriers due to proteins' hydrophilic characteristic (Krochta, 1992). Thus, lipids have been combined into protein-based films to produce films with improved moisture barrier properties (Gennadios et al., 1994). The following reviews the influence of lipids on barrier and mechanical properties of protein/lipid emulsion edible films.

1.2.1 Water vapor permeability

Gontard et al. (1994) reported water vapor permeability (WVP) of wheat gluten/ lipid emulsion films with various lipid materials, such as soya lecithin, acetylated monoacylglycerol, beeswax, carnauba wax and paraffin wax. A lipid material was added into the film forming solution and mixed together, then warmed until it melted. Addition of soya lecithin and acetylated monoacylglycerol (both polar lipids) increased WVP of the wheat gluten/lipid emulsion films. On the contrary, beeswax, carnauba wax and paraffin

wax (all posses strong hydrophobic characteristics) contributed to improve resistance of the films to water vapor transport. These results were also related to melting point differences among lipids. Melting points of soya lecithin and acetylated monoacylglycerol are 20°C, while beeswax, carnauba wax, and refined paraffin wax have higher melting points, 61°C, 70°C, and 75°C, respectively. They concluded that use of lipids with high melting point resulted in decreased WVP of wheat gluten/lipid emulsion films.

Avena-Bustillos and Krochta (1993) determined WVP of acetylated monoacylglycerol- and beeswax-added sodium caseinate-based edible films. Beeswax was more effective than acetylated monoacylglycerol in reduction of WVP. Acetylated monoacylglycerol-added sodium caseinate films had almost twice the permeability of beeswax-added sodium caseinate films. Chick (1998) studied the effect of carnauba and candelilla wax on WVP of lactic acid casein-based films. WVP was significantly decreased as wax concentration increased. Candelilla wax was more effective in reducing WVP of the films than carnauba wax.

McHugh and Krochta (1994c) investigated effects of incorporating fatty acids into whey protein-based edible films to reduce their WVP. Incorporation of fatty acids significantly decreased WVP of whey protein/lipid emulsion films. Increased chain length of fatty acids lowered WVP of the films. WVP of palmitic acid (C_{16}) and myristic acid (C_{14}) incorporated whey protein-based films were 19.2 and 23.8 $g \cdot mm/m^2 \cdot day \cdot kPa$, respectively. Shellhammer and Krochta (1997) investigated WVP of whey protein/lipid emulsion films containing beeswax, candelilla wax, carnauba wax, and a high-melting fraction of anhydrous milk fat. Each lipid was added at same level, 40% of the film dry weight, beeswax and milk fat fraction distinctively decreased WVP of the whey

protein/lipid emulsion films from 45.4 to 10.8 and 21.9 $g \cdot mm/m^2 \cdot day \cdot kPa$, respectively. With all lipid types, WVP of the films were significantly reduced as lipid concentration increased. They concluded that the lipid type and concentration were important in controlling WVP of the whey protein/lipid emulsion films.

1.2.2 Oxygen permeability

Unlike the results observed in WVP of protein/lipid emulsion films, no specific trends were observed in oxygen permeability (OP). Gennadios et al. (1993c) investigated the effect of acetylated monoacylglycerol on OP of wheat gluten/lipid emulsion films. They reported that acetylated monoacylglycerols significantly lowered OP of the films by about 30% than that of the control film. McHugh (1996) reported that OP of whey protein/beeswax emulsion films at two relative humidity testing conditions of 46 and 70% were 8.6 and 101.1 $cm^3 \cdot \mu m/m^2 \cdot day \cdot kPa$, respectively. OP of whey protein films with no lipid at the same relative humidity conditions were 1.5 and 42.3 $cm^3 \cdot \mu m/m^2 \cdot day \cdot kPa$, respectively. Although no statistical comparisons were made, it is apparent that addition of beeswax increased OP of whey protein-based films at both relative humidity conditions. Chick (1998) studied effect of carnauba and candelilla waxes on OP of lactic acid casein-based films. Reportedly, concentration of carnauba and candelilla waxes had no significant effect on OP of lactic acid casein/lipid emulsion films and neither did the wax type.

1.2.3 Mechanical properties

Lai et al. (1997) prepared zein/lipid emulsion films with palmitic acid (0, 0.25, 0.5, 0.75 and 1g of palmitic acid per gram of zein), and reported on their tensile strength (TS). Films with no palmitic acid showed the lowest TS. Addition of palmitic acid increased TS, however when it was added at more than 0.75g/g of zein TS decreased continuously as the concentration of palmitic acid increased. Santosa and Padua (1999) investigated mechanical properties of zein/lipid emulsion films with oleic acid. Oleic acid was added into zein film-forming solutions at various ratios. TS of the films decreased from 9.4 to 2.2 MPa as oleic acid levels increased from 0.5 to 1.0 g/g of zein. On the other hand, addition of oleic acid increased the film's percent elongation (%E), which is a measure of film's ability to stretch. However, excess oleic acid decreased %E, probably due to weakened structure of the films. Rhim et al. (1999a) investigated the effects of lauric and stearic acids on TS of soy protein/lipid emulsion films. They reported decreased TS and %E upon incorporation of these fatty acids.

Banerjee and Chen (1995) investigated TS and %E of whey protein/lipid emulsion films. Acetylated monoacylglycerol was added into whey protein concentrate and isolate film-forming solutions at 1:2 ratio (w/w, lipid:protein). TS and %E of acetylated monoacylglycerol-added whey protein-based films were significantly lowered in both whey protein types compared to films without lipids. Whey protein concentrate-based films had 3.4 MPa and 20.8 % of TS and %E, respectively. These values were decreased to 1.1 MPa and 13.6 % after addition of acetylated monoacylglycerol. A similar trend was observed in the values of whey protein isolate-based films. Shellhammer and Krochta (1997) studied effects of beeswax, candelilla wax, carnauba wax, and a high-melting

fraction of anhydrous milk fat on TS and %E of whey protein-based films. Lipid type and concentration affected TS of the films. Increasing lipid levels linearly decreased TS of the films for all lipid types. However, carnauba wax-added films were the strongest at all concentrations. No significants effect of the lipid type and concentration on %E of the films were observed except for the milk fat-added films. As the concentration of milk fat increased, %E of the films increased significantly, probably resulting from plasticizing effects of unsaturated and low molecular weight triacylglycerols in the milk fat. Overall, incorporation of lipids into protein-based films altered films mechanical properties. Type of lipid and concentration seemed to be the most important factors affecting mechanical properties of protein/lipid emulsion films.

1.3 COMPOSITION AND PROPERTIES OF COMPONENTS USED IN EDIBLE FILMS.

1.3.1 Whey proteins

Cheese whey is the liquid remaining after the precipitation and removal of casein during cheese manufacturing. About 9 kg of whey is generated to produce 1 kg of cheese (Kosikowski, 1979). Liquid whey represents 85-95% of the milk volume, and contains lactose, soluble proteins, lipids and mineral salts. Among them soluble proteins make up 0.6-0.8% (w/v) of liquid whey (Siso, 1996). Cheese whey is typically processed to concentrate the whey proteins. Whey proteins are sold commercially in various forms as ingredients to be used in baked goods, processed meats etc. due to their desirable functional properties. Following are the manufacturing processes for whey protein products.

1.3.1.1 Manufacture of whey proteins

Cheese whey tends to spoil easily, due to its low solids and high moisture content and presence of cheese starter organisms. Thus, moisture is removed to prolong its shelf life. This is achieved by high velocity and low temperature spray drying. High temperature (>75°C) is avoided to prevent denaturation of whey proteins, which may alter their functional properties (Renyard and Whitehead, 1992; Morr and Ha, 1993). Composition of whey powders varies with the manufacturing process. Whey powder can be obtained with reverse osmosis by removing water. This has 13% protein, 1% fat, 76% lactose and 10% ash. The 35% whey protein concentrate (WPC) contains 35% protein, 4% fat, 53% lactose and 8% ash. The 50% WPC has 53% protein, 5% fat, 35% lactose and 7% ash. In 80% protein WPC, lactose is reduced to 7%, and the fat and ash range from 4 to 7%. Whey protein isolate (WPI) has more than 90% protein with less than 1% fat, and lactose and ash vary between 1 to 4% (Early, 1992; Huffman, 1996).

To make a WPC, whey is pasteurized and clarified. The clarifier (a large scale centrifuge) purifies whey by removing small particles of cheese and casein. Then, ultrafiltration (UF) physically removes lactose and minerals, and concentrates whey protein and fat. The membrane molecular weight (MW) cut-off is typically 20,000, thus smaller particles, such as water, salts and lactose are readily removed. Following spray drying a fine white powder is produced. Diafiltration (DF) can further concentrate the whey protein up to 80%. This membrane process involves applying a water stream to wash out lactose and minerals (Morr and Ha, 1993; Huffman, 1996).

To produce a WPI, two additional processing steps are required. Microfiltration eliminates fat, and lactose hydrolysis removes the lactose. Both processes are followed by

UF and DF to make a low-fat, low-lactose WPI (Huffman, 1996). Ion-exchange is another way to obtain a WPI. This process is a pretreatment prior to UF to produce WPI (Early, 1992; Morr and Ha, 1993). An outline of the whey protein manufacturing process is given in Figure 1.1.

1.3.1.2 Protein fraction of whey

Whey proteins represent 15-25% of the total milk protein. Whey proteins consist of β-lactoglobulin, α-lactalbumin, bovine serum albumin, immunoglobulins, and proteose peptone (Brunner, 1981). Whey proteins also include a number of enzymes such as alkaline phosphatase, lactoperoxidase, sulfhydryl oxidase and catalase, and metalloproteins, like lactoferrin (Cayot and Lorient, 1997).

1.3.1.2.1 β-Lactoglobulin — β-lactoglobulin (β-Lg) makes up approximately 50% of the whey proteins (Cayot and Lorient, 1997), and 7-12% of the total milk protein (Brunner, 1981). β-Lg is a globular protein with a molecular mass of approximately 18,300 daltons (Eigel et al., 1984). It has 162 amino acid residues and contains one free sulfhydryl group and two disulfide bonds. The amino acid composition of β-Lg is provided in Appendix I. Seven genetic variants of β-Lg have been characterized, among them A and B are the main variants. The A variant has Asp and Val, and B variant has Gly and Ala at residue 64 and 118, respectively (Cayot and Lorient, 1997). Bovine β-Lg shows a high degree of organization in the secondary and tertiary structures with 50% β sheets, 15% α helix, and 15-20% β turns. Its compact structure is a result of two disulfide bonds and stacked nine β sheets (Creamer et al., 1983). The free Cys 121

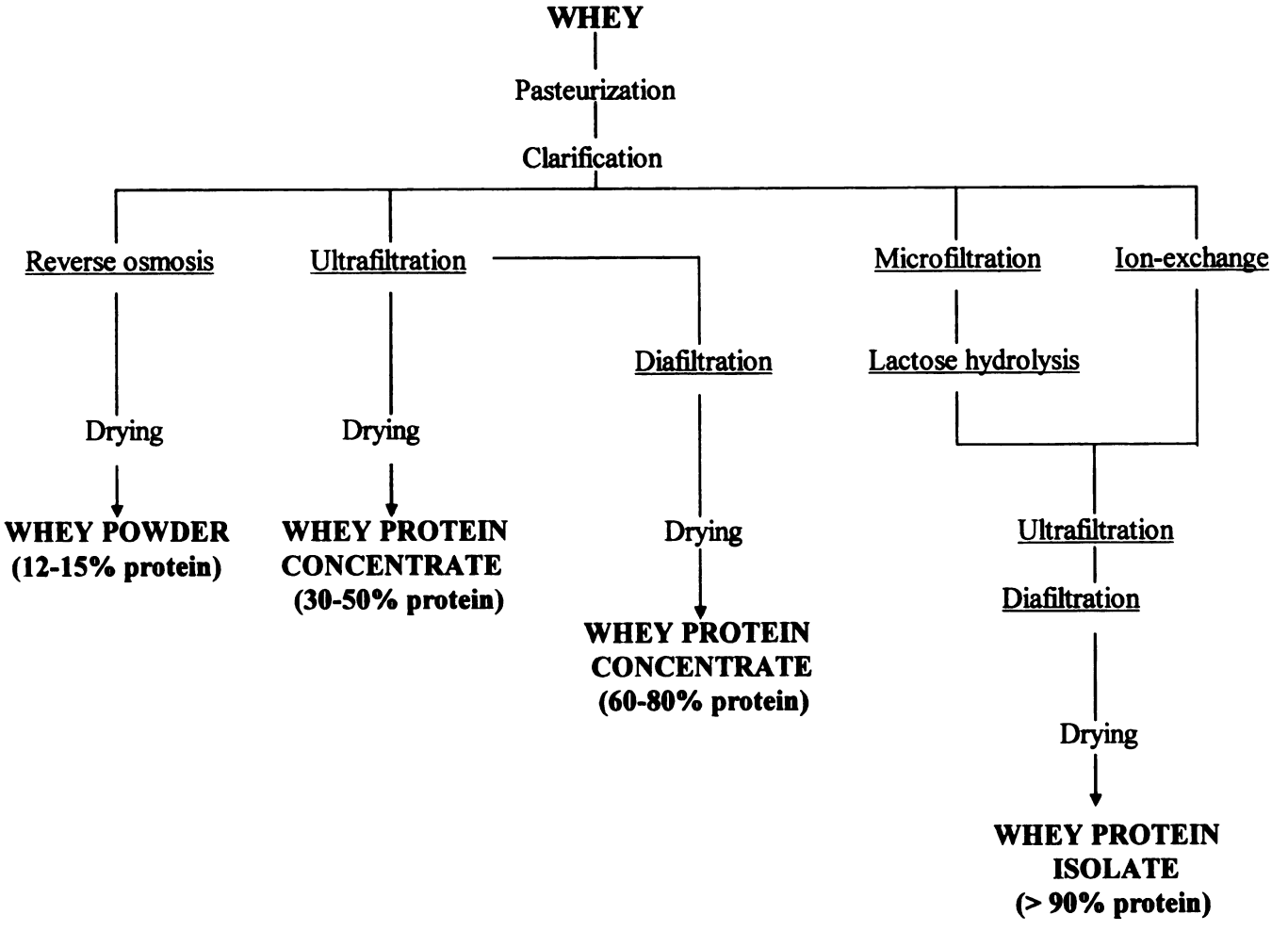


Figure 1.1. Manufacturing process of whey proteins (Early, 1992; Huffman, 1996).

residue is located at the sheet-helix interface, and two disulfide bonds are formed Cys 66 to Cys 160 and Cys 106 to Cys 119 (Wong et al., 1996). Most of non-polar residues in β-Lg are buried in the protein's interior, forming a hydrophobic pocket, and a majority of the polar groups are exposed at the surface (Cayot and Lorient, 1997).

Bovine β-Lg exhibits pH-dependent association-dissociation behavior. β-Lg generally exits as a dimer at pH 5-8. At pH 3-5 the dimers tend to form octamers. Below pH 2 and above pH 8, β-Lg dissociates into monomers (Passen et al., 1985; Monaco et al., 1987). Thermal denaturation and aggregation of β-Lg occur above 65°C. Denaturation of β-Lg initiates with exposure of the reactive Cys 121. This reversible conformational change leads sulfhydryl/disulfide exchange to form disulfide bonds then association of unfolded species through hydrophobically bonded aggregates. This aggregation is irreversible and displays time and temperature dependency (Gough and Jenness, 1962; Sawyer et al., 1971; Gezimati et al., 1996).

1.3.1.2.2 α-Lactalbumin — α-lactalbumin (α-La) represents approximately 19% of the whey proteins (Cayot and Lorient, 1997), and 2-5% of the total milk protein (Brunner, 1981). It has four disulfide groups with a molecular mass of 14,147 daltons for genetic variant A and 14,175 daltons for B. α-La contains 123 amino acid residues, and the variants A and B differ at residue position 10; Gln for A and Arg for B (Brew et al., 1970; Eigel et al., 1984). The amino acid composition of α-La is provided in Appendix I.

α-La exhibits a very low tendency of organized secondary structure. It consists of 30% α helix and 9% β sheets (Alexandrescu et al., 1993). The α-La molecule shows

ellipsoidal shape, and a deep cleft divides the molecule into two lobes. One side of the lobe comprises of four helices, and the other lobe contains two β strands and loop-like chain (Wong et al., 1996). Heat treated (at 80°C) α-La recovers its structure after heat treatment; this reversibility makes it more heat resistant than β-Lg. The apparent difference in thermal aggregation behavior between α-La (reversible) and β-Lg (irreversible) is likely to be based on the fact that β-Lg has self initiation ability of sulfhydryl/disulfide exchange reaction, but not α-La. However, the aggregation rate of α-La is increased when α-La is heated in the presence of β-Lg. This aggregation occurs through disulfide bonded copolymer formation between α-La and β-Lg (Hines and Foegeding, 1993; Gezimati et al., 1997).

1.3.1.2.3 Bovine serum albumin — Bovine serum albumin (BSA) makes up about 5% of whey proteins (Cayot and Lorient, 1997), and 0.7-1.3% of the total milk proteins (Brunner, 1981). BSA is a carrier protein, functioning to transport nonpolar molecules in biological fluids (Whitney et al., 1976). The BSA molecule is a single peptide chain with 582 amino acid residues with a molecular mass of approximately 66,000 daltons. The N-terminal amino acid residue is Asp and Ala is the C-terminal. It appears to be that the BSA molecule has an ellipsoidal shape and the N-terminal region is more compact than the C-terminal region. It has 17 intramolecular disulfide bonds with one free sulfhydryl group at residue 34 (Eigel et al., 1984). BSA can be denatured by heating or by acid or base treatment (Cayot and Lorient, 1997). BSA shows very similar denaturation behavior to that of β-Lg. Upon heating, BSA forms disulfide bonded-polymers and aggregates through hydrophobic interactions (Gezimati et al., 1996).

1.3.1.2.4 Immunoglobulins and proteose-peptone — Immunoglobulins form family of high molecular weight proteins, such as IgG_1, IgG_2, IgA, IgM and IgE, with antibody properties. Immunoglobulins represent 1.4 -3.1% of total milk proteins (Brunner, 1981), and make up around 13% of whey proteins (Cayot and Lorient, 1997). Immunoglobulins have molecular mass of 15,000 to 1,000,000 daltons (Whitney et al., 1976; Eigel et al., 1984). The immunoglobulin molecule consists of two light polypeptide chains (~20,000 daltons), and two heavy polypeptide chains (~50,000 to 70,000 daltons). The light and heavy polypeptide chains are cross-linked by disulfide bonds (Whitney et al., 1976; Brunner, 1981). About 80% of immunoglobulins in milk are IgGs (Eigel et al., 1984).

Proteose-peptones (PP) account for 2-4% of total milk proteins, and 10% of whey proteins. Their molecular masses range from 4,000 to 40,000 daltons (Brunner, 1981). The PP components remain soluble after precipitation of casein at pH 4.6, and heat coagulation of the β-Lg and α-La at 95°C for 30 min. Thus, the PP fraction is defined as a mixture of acid-soluble and heat-stable phosphoglycoproteins (Whitney et al., 1976; Girardet and Linden, 1996).

1.3.2 Lipids

Candelilla wax (CW) is a vegetable wax from a reed-like plant (*Euphorbia Antisiphilitica, Euphorbia Cerifera, and Pedilanthus Pavanois*), which grows in northwestern Mexico and southern Texas. When the plant reaches a height of one to three feet, stalks are uprooted and utilized. The plant is immersed into boiling water containing sulfuric acid and wax is extracted, strained, and cooked to remove excess water (Bennett, 1975). The wax consists of 50-51% hydrocarbons and 29% wax esters, with

the remainder consisting mainly of alcohols and free acids. The melting point of CW ranges between 66 and 71°C. CW exists as hard, brittle, and lustrous granules and has a honey-like aromatic odor (Bennett, 1975). CW belongs to the non-polar lipid class; it is insoluble in water and has high hydrophobicity like other waxes (Callegrain et al., 1997). CW is GRAS (generally-recognized-as-safe) and is approved by the Food & Drug Administration (FDA) for use in fruit and vegetable coatings, confections and beverages. There are no limitations in usage of CW other than good manufacturing practice (CFR, 184.1976; Hernandez and Baker, 1991; Hernandez, 1994; Baldwin et al., 1997)

Butter fat (BF) or milk fat accounts for 3.5-3.8% of bovine milk. The milk fat exists in milk as small fat globules emulsion dispersed in the aqueous phase. The fat globules range from 2 to 10μm in diameter. Total milk lipids contain 97-98% triacylglycerols. The remaining lipids are di- and monoacylglycerols, phospholipids, free fatty acids, and cholesterol and its esters. Triacylglycerol consists primarily of oleic, stearic and palmitic acids with smaller amount of triacylglycerols of butyric, caproic, caprylic and capric acids (Swaisgood, 1985; Sax and Lewis, 1987; Igoe, 1989; Muir, 1992). Milk fat triacylglycerols contain approximately 10-15% high melting, 30-45% middle melting and 35-55% low melting acylglycerols, and their melting points are around 20-40°C, 0-20°C and 0°C, respectively. Since the main components of BF are triacylglycerols, BF is classified as a polar lipid (Lane, 1992; Callegrain et al., 1997). Commercial butter is required to contain at least 80% fat by weight and maximum 16% in moisture (Lane, 1992).

1.3.3 Plasticizers

Sorbitol (D-glucitol) was discovered from the ripe berries of mountain ash (*Sorbus aucuparia L.*) in 1872. It can be produced by high-pressure hydrogenation or electrolytic reduction of D-glucose, or by catalytic hydrogenation of dextrose. The molecular weight of sorbitol is 182.17 daltons, and it consists of a 6 carbon chain with 6 hydroxyl groups ($C_6H_{14}O_6$). Its structure is shown in Appendix II. Sorbitol is stable and chemically unreactive (Budavari et al., 1989). Sorbitol is a polyol (polyhydric alcohol) with good solubility in water and poor solubility in oil. It is slightly soluble in methanol, ethanol, acetic acid, phenol and acetamide, and almost insoluble in most other organic solvents. The relative sweetness of sorbitol is approximately 60 % that of sucrose. Sorbitol is a white, odorless and hygroscopic crystalline powder. Hydrated sorbitol crystals melt below 100°C, while anhydrous sorbitol melts at 110-112°C. Sorbitol is approved by FDA for food use as a sweetener, humectant, emulsifier, thickener, anticaking agent and dietary supplement (Sax and Lewis, 1987; Budavari et al., 1989; Igoe, 1989).

Glycerol is a three-carbon molecule with one hydroxyl group ($C_3H_8O_3$), and its structure is shown in Appendix II. It has a molecular weight of 92.09 daltons. It is a colorless, odorless clear syrupy liquid with high solubility of 71g per 100g of water at 25°C. It is insoluble in ether, benzene, and chloroform. Glycerol has medium to high hygroscopicity. Melting temperature of glycerol is 17.8°C. It is about 60% as sweet as sucrose (Budavari et al., 1989; Igoe, 1989). Glycerol is a byproduct of soap manufacture. It can be produced by catalytic hydrogenation of carbohydrates or isomerization of propylene oxide to allyl alcohol, which is then hydrolyzed to glycerol. Glycerol is used as

a humectant in pharmaceuticals, a sweetener in confectioneries, fermentation nutrient in antibiotic production, and as a plasticizer for regenerated cellulose (Sax and Lewis, 1987).

1.4 PROPERTIES OF FILMS IMPORTANT FOR FOOD APPLICATIONS

1.4.1 Barrier properties

Food product quality depends on loss of or exposure to vapor and gases, such as water, oxygen, carbon dioxide, or volatile compounds. Functions of edible films include providing barriers to the transfer of water, gas, or solute (salts, pigment, or lipids). Therefore, the measurements of permeability values of edible films are important in determining their performances as packaging materials (Debeaufort et al., 1998). Water vapor permeability (WVP) and oxygen permeability (OP) are the most commonly studied barrier properties of edible films.

Permeability is defined as transmission of vapor or gases through polymer materials. This permeation involves adsorption of the vapor or gas into the polymer surface, its diffusion through the polymer, and its desorption through the opposite surface by evaporation (Sperling, 1992). A diagram of the permeability model is shown in Figure 1.2. This is expressed by the following equation:

$$P = D \times S \qquad (1)$$

P is the permeability coefficient and commonly called permeability, D is the diffusion coefficient, and S is the solubility coefficient (Giacin and Hernandez, 1997). The diffusion coefficient refers to the speed that the molecules move into the polymer. Diffusion movement occurs from the side of the polymer that is in contact with high concentration

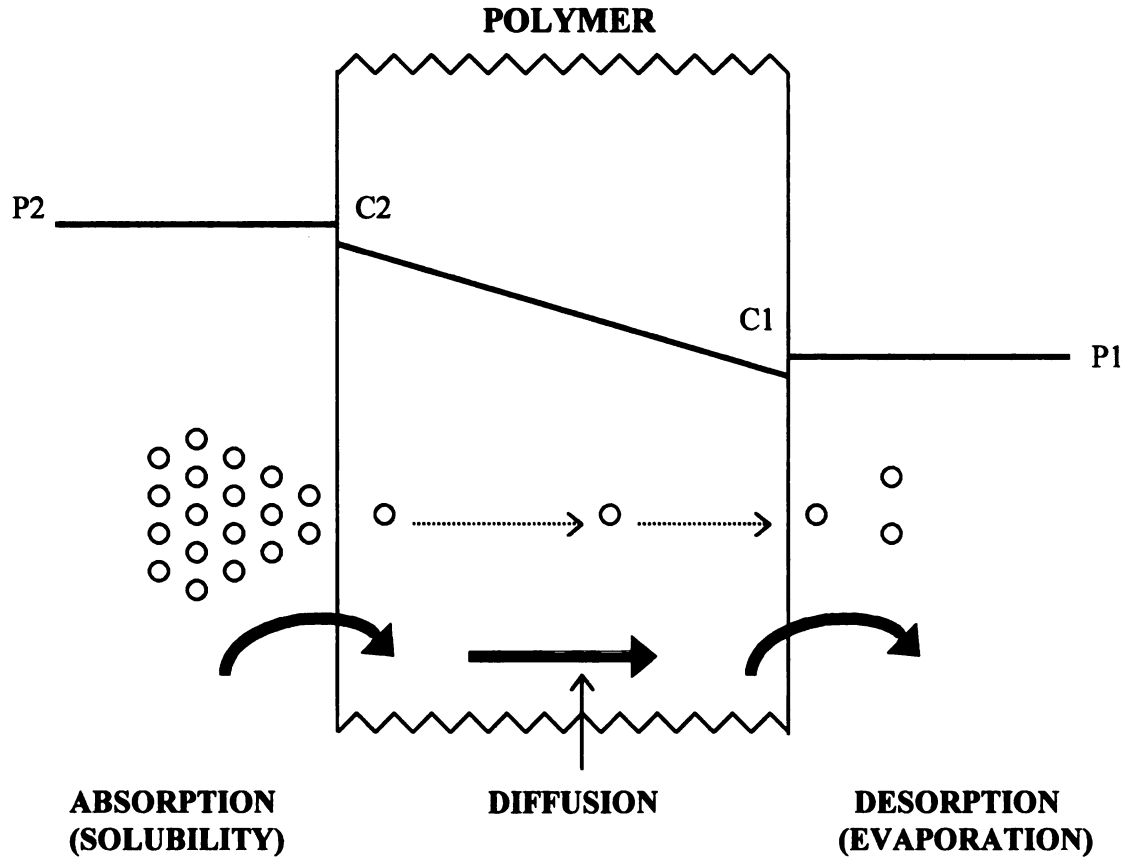


Figure 1.2. Permeability model for gas or vapor transfer through a barrier polymer (Giacin and Hernandez, 1997).

or partial pressure of permeant to the side that is in contact with a low concentration of permeant. The diffusion process further depends on the size, shape and polarity of the diffusing molecules, and the structure and characteristics of the films. The solubility coefficient is the number of permeant molecules that are diffusing. Solubility coefficient refers to the solubility parameters of vapors or gases to the particular polymer. The solubility influences dissolution and evaporation of the permeating substance at the interface of the film. A low permeability is the result of a low diffusion coefficient or a low solubility coefficient or both. The permeation test for vapor or gas is run until steady state is reached (Guilbert and Biquet, 1996; Delassus, 1997).

Water vapor transmission rate (WVTR) is the rate of water vapor flow to the surface, under steady-state conditions, per unit area. Water vapor permeance is the ratio of a barrier's WVTR to the vapor pressure difference between the two surfaces. Water vapor permeability (WVP) is the product of the permeance and the thickness of the film (ASTM, 1997). WVTR can be tested using an infrared or coulometric sensors; spectrophotometric, gas chromatograhic or gravimetric techniques.

Only an infrared sensor method, ASTM F-1249 "*Standard test method for water vapor transmission rate through plastic film and sheeting using a modulated infrared sensor*" (ASTM, 1997), is reviewed here since this method was the one adopted to measure WVP of the films developed in this present research. The operation diagram of an infrared sensor method is shown in Figure 1.3. First, desired temperature and humidity conditions are set. Test film is placed in a diffusion cell to separate a dry chamber and a wet chamber. Then, the dry chamber side is swept with dry air, which carries diffused water vapor through the test film into an infrared sensor. The sensor detects infrared

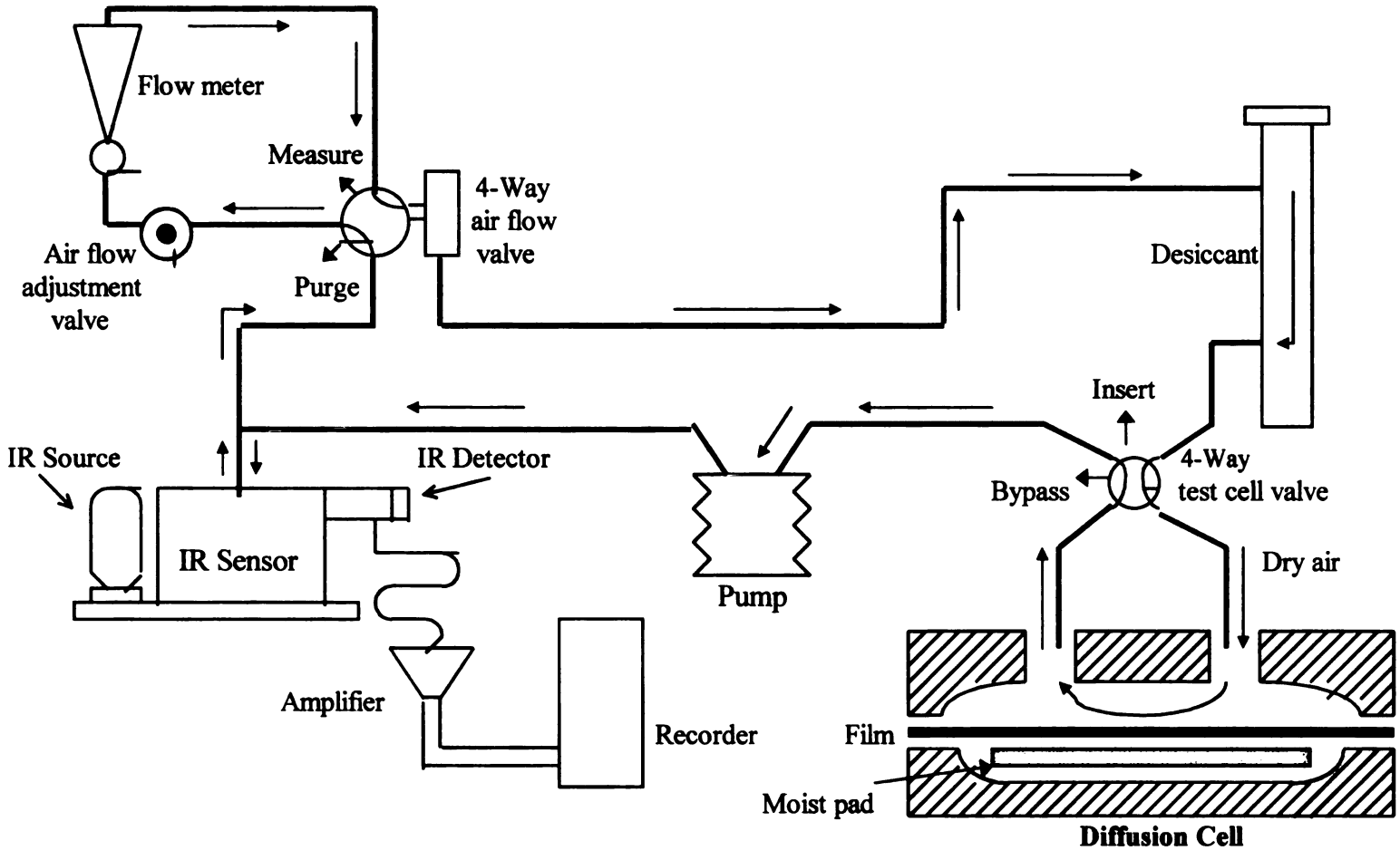


Figure 1.3. Diagram of the water vapor permeability measurement using the infrared sensor technique (ASTM, 1997).

energy absorbed by the water vapor and yields an electrical signal, which is proportional to water vapor concentration. WVTR is obtained by the equation:

$$WVTR = C \times (ES - EO) \quad (2)$$

Where, C = a calibration factor

ES = equilibrium voltage obtained with the test film

EO = steady-state voltage produced by dry air

This value is used to calculate water vapor permeance:

$$Permeance = WVTR / \Delta p \quad (3)$$

Where, WVTR = water vapor transmission rate of the test film

Δp = water vapor partial pressure gradient across the test film

Then, permeability is calculate as following:

$$WVP = permeance \times d \quad (4)$$

Where, WVP = water vapor permeability of the test film

d = thickness of the test film

WVP can be expressed as the $g \cdot mm/m^2 \cdot day \cdot kPa$ and since the permeance is the function of relative humidity (RH) and temperature, the test condition must be stated.

Oxygen transmission rate (OTR) is the quantity of oxygen that passes through a unit area of a test film per unit time. Oxygen permeance is the ratio of the OTR to the difference between the partial pressure of oxygen across the test film. Oxygen permeability (OP) is the product of permeance and the thickness. Coulometric sensor technique is the most commonly used method to determine OP of films, and this method was also adopted in this present study.

ASTM D-3985 "*Standard test method for oxygen gas transmission rate through plastic film and sheeting using a coulometric sensor*" (ASTM, 1997) can be used to obtain OTR. A test film is placed between two chambers at ambient atmospheric pressure. OTR is determined after the test film is equilibrated in a dry condition, which is less than 1% RH. One side of the chamber is purged with nitrogen and the other side by air containing 21% of oxygen. Nitrogen gas carries permeated oxygen through the test film into the coulometric sensor, which produces an electrical current. This electrical current is proportional to the amount of oxygen flowing into the sensor per unit time. Operation diagram of the measurement of OTR is given in Figure 1.4. To obtain the OP, thickness of the film and the partial pressure are taken into account shown by the equation:

$$OP = (OTR \times d) / \Delta p \qquad (5)$$

where d is thickness, and Δp is partial pressure of oxygen. OP can be expressed as the $cm^3 \cdot \mu m/m^2 \cdot day \cdot kPa$ and the test condition must be stated.

1.4.2 Mechanical properties

Edible films need to have a certain degree of mechanical strength with sufficient flexibility to resist breakage during distribution and handling of packaged products. Thus determination of mechanical properties is necessary to provide information to set appropriate storage and handling requirement of edible films (Gontard and Guilbert, 1994). Various mechanical properties of edible films have been evaluated, such as tensile strength, puncture strength, extensibility to puncture, torsion resistance, elasticity, elongation at break, elongation at yield, etc. (Guilbert and Biquet, 1996). Among them,

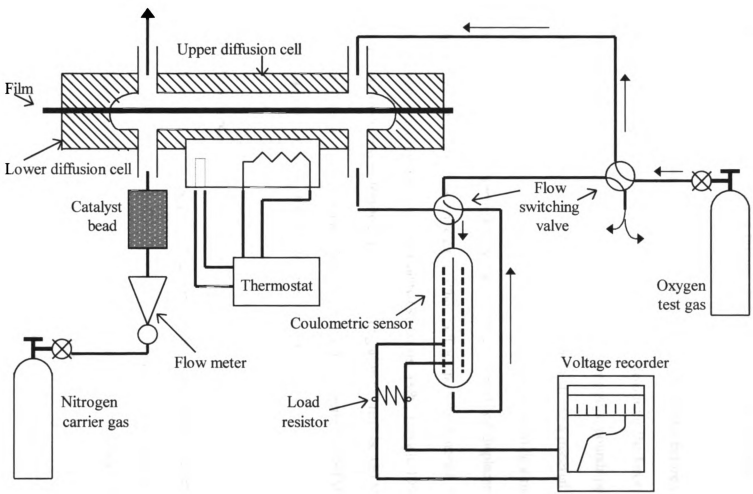


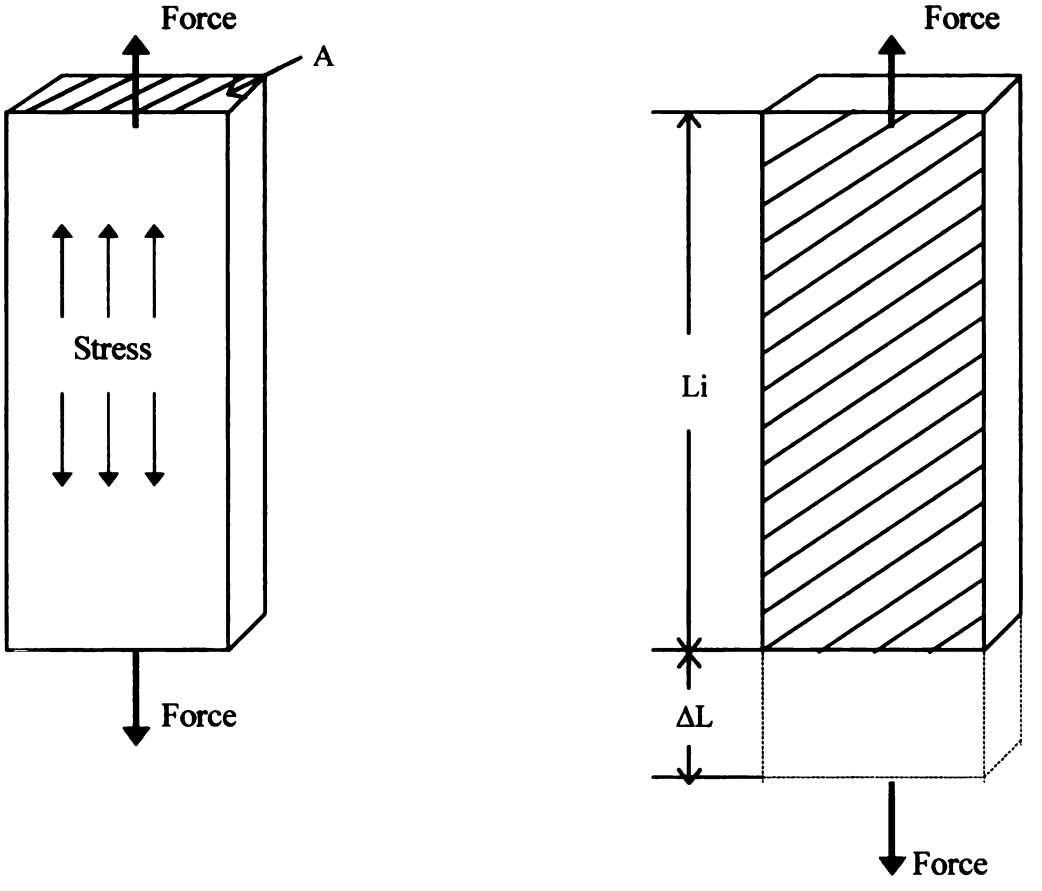
Figure 1.4. Diagram of the oxygen permeability measurement using the coulometric sensor technique (ASTM, 1997).

tensile strength (TS) and percent elongation (%E) are the most common mechanical properties to evaluate edible films and to predict their performance as packaging materials (Gennadios et al., 1994).

TS refers to the maximum tensile stress that a material can sustain before the onset of permanent deformation or failure. This is determined by measuring the force per unit area (F/A) while the material is pulled apart, and is usually expressed in pounds per square inch (lbs/in^2) or megapascals (MPa). TS is an indicator of how strong a material is. %E is the deformation caused by stretching and fractional increase in length while a material is stressed in tension. %E is determined by measuring the length of displacement per original length (ΔL / Li, reported as percentage) while pulling apart the material. %E is an indicator of the material's toughness and flexibility (Symonds, 1989; Koyich, 1992). The established method to determine tensile strength and elongation at break is ASTM D-882, "*Standard test method for tensile properties of thin plastic sheeting*" (ASTM, 1997). Diagram of mechanical properties measurement is shown in Figure 1.5.

1.4.3 Thermal properties

In considering thermoplastic processing, for instance heat-sealing or extrusion, for edible films, one of the most important limitations is the lack of information on their thermal properties. Thus, exploiting thermal properties of biopolymer-based films and their components is crucial in the development of edible packaging materials. Several different thermal analysis techniques are available that measure temperature of transition, heat capacity change, and energies of transition or enthalpic change (ΔH) of processing materials, such as differential scanning calorimetry (DSC), differential thermal analysis



a) Tensile strength

b) Percent elongation

Figure 1.5. Diagram of mechanical properties measurement (Symonds, 1989). (a) Tensile strength; (b) Percent elongation. A = cross-sectional area; Li = original gage length; ΔL = change in original gage length .

(DTA), thermogravimetric analysis (TGA), thermomechanical analysis (TMA) and dynamic mechanical thermal analysis (DTMA). Among them, DSC is the most common thermal analysis technique. DSC measures the differential temperature or heat flow to or from a sample versus a reference material (Figure 1.6). This can be plotted as a function of temperature or time (Davis, 1994).

Thermoplastic processing is possible when the raw materials have a wide plastic range between room temperature and its degradation temperature (T_d). In general, film production is carried out at a temperature moderately above glass transition temperature (T_g), or at melting temperature (T_m) of the polymer (Hernandez, 1997; Metzger, 1997).

T_g is the temperature where the reversible change occurs in amorphous polymer or in amorphous regions of partially crystalline polymer from (or to) a viscous and rubbery condition to (or from) a relatively brittle one. The T_g of polymers range from -25°C to 365°C (ASTM, 1997; Hernandez, 1997). Heating amorphous thermoplastic biopolymers above their T_g results in soft and rubbery materials, which allows them to be shaped as packaging materials. Cooling at room temperature reconverts the rubbery materials into a glassy product with desired shape (Cuq, 1997a; Krochta and De Mulder-Johnston, 1997). T_m is the temperature of molten polymers. Most semicrystalline polymers have a T_m range instead of having a sharp melting peak. Amorphous polymers don't have a T_m. The T_m of materials could range from 2°C to 455°C depending on the polymer. T_d is the point where thermal decomposition of polymers occurs. Thermal degradation of polymers is the breaking of bonds by heat in the absence of oxygen. As the temperature increases, chemical bonds with low energy values will be broken first, and thermally stable bonds will

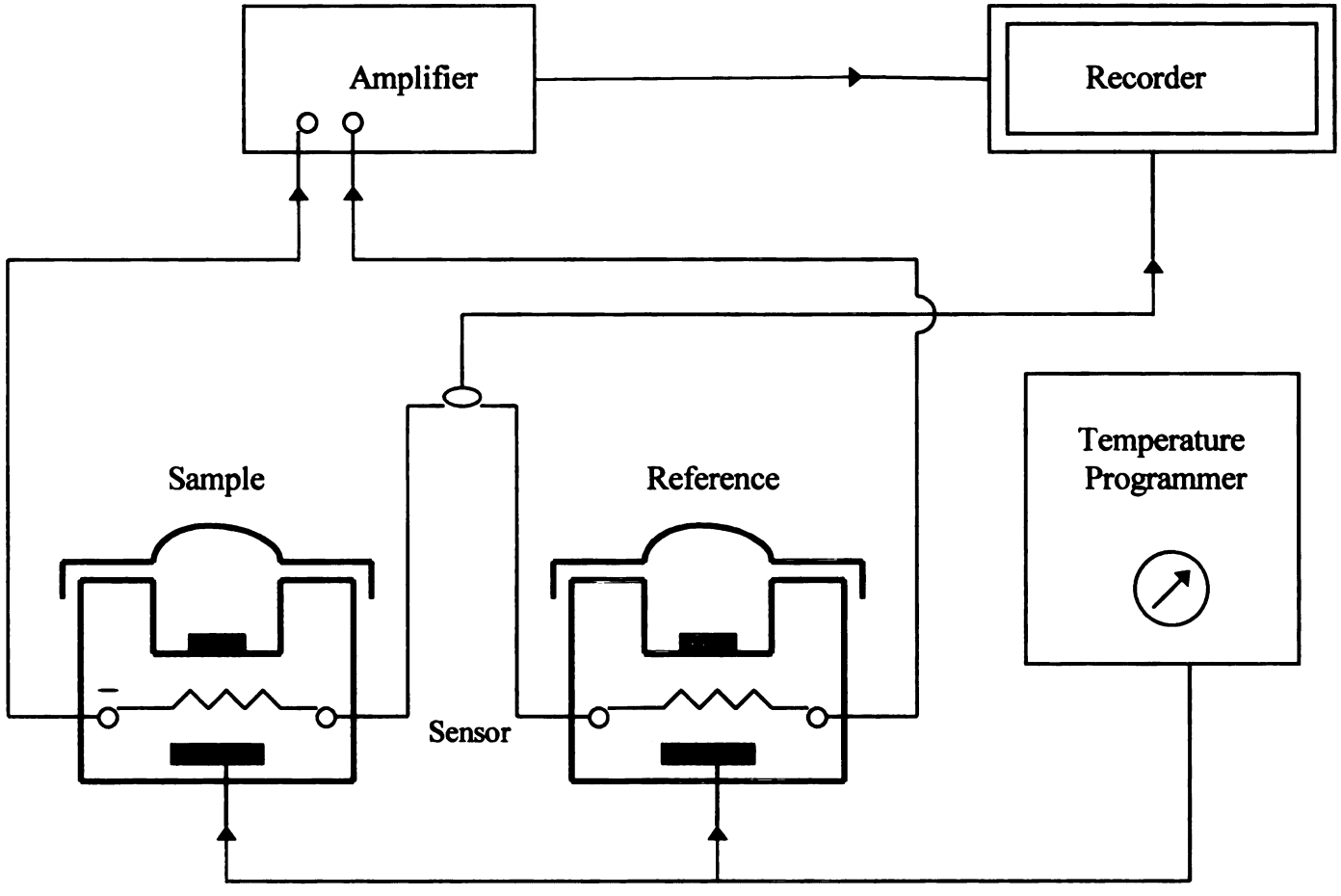


Figure 1.6. Schematic diagram of the differential scanning calorimetry instrument (Cummings, 1985; Davis, 1994).

resist thermal degradation and require higher energy to dissociate them. Thus T_d can occur as multiple peaks (Throne, 1986; ASTM, 1997; Hernandez, 1997).

ASTM D-3418 "*Standard test method for transition temperatures of polymers by thermal analysis*" (ASTM, 1997) covers determination of transition temperatures of polymers by DSC. This standard method also shows determination of onset (T_o), peak (T_p), and end (T_e) temperatures of *first-order* transition. T_m is a *first-order* transition which exhibits a discontinuity with temperature. On the other hand, T_g is a *second-order* transition which exhibits a temperature continuity (Rosen, 1982).

Cuq et al. (1997a) studied thermal properties of myofibrillar proteins and myofibrillar protein-based edible films. They reported that T_g of the myofibrillar proteins were drastically decreased when it was formed as dried films, from 215-250°C to 130-185°C, respectively. They explained that this larger decrease (> 75°C) in T_g is due to addition of glycerol. Similar trends were observed with zein, corn gluten, and gelatin-based edible films upon incorporation of plasticizers (O'Donnell et al., 1997; Di Gioia and Guilbert, 1999; Menegalli et al., 1999). Plasticizers separate interactions between polymers causing reduction of their resistance to applied stress, which results in reduced T_g (Throne, 1986). The presence of hydrophilic plasticizers in films also decreases the T_m by enhancing molecular mobility of the film structure (Gutiérrez-Rocca and McGinity, 1994; Aravanitoyannis et al., 1998).

1.4.4 Sealing properties

Sealability of the edible films is an appealing attribute, and important in the development of pouches or sachets. It is desirable to have materials that are not only

edible but also heat-sealable from the viewpoint of edible-packaging operation. There are different heat-sealing techniques such as bar sealing, band sealing, impulse sealing, hot wire or knife sealing, gas sealing, contact sealing, hot-melt sealing, and pneumatic sealing, etc. (Young, 1997). Although there are many different methods for heat-sealing, the basic principles remain the same. Surface regions of polymer melt when heat is applied. The bonding of two polymer surfaces is initiated by bringing them together while they are in partially molten state. With the application of pressure, polymer-chain segments from opposite sides of the interface diffuse across the interface, and molecular entanglements are induced between the melted surface. After cooling, a heat sealed joint of the two polymer surfaces is completed (Stehling and Meka, 1994).

The effect of heat-sealing depends on sealing temperature, pressure, and dwell time. To achieve sealing with sufficient strength, the surface must be pressed together an adequate length of time and pressure so the polymer chains can diffuse across the interface and form bridged bonds (Meka and Stehling, 1994; Mueller et al., 1998). However, the important seal properties of the films such as seal strength, toughness, and appearance are much affected by the sealing temperature. Appropriate sealing temperature or seal range is determined according to data obtained from thermal analysis of the polymer. Seal range is the range of temperature in which effective seal can be obtained at constant dwell time and pressure, and the highest sealing temperature should be determined at which a seal can be obtained without deterioration of the seal or the polymer structure (Martin, 1986).

ASTM F-88 "*Standard test method for seal strength of flexible barrier materials*" (ASTM, 1997) is designed to provide a standard seal strength test for packaging materials and adopted in this present study. Studies have demonstrated heat sealability of

biopolymer-based films from casein, carrageenan and soy protein (Georgevits, 1967; Ninomiya et al., 1990; Rhim et al., 1999a). However, there are no reports on the seal properties of whey protein-based edible films.

The sealed joints studied by seal strength test provides macroscopic information on the bond strength, however it doesn't give information on the nature of the sealing mechanism or on the chemical characteristics of the sealed surfaces. Thus, a surface analytical technique that provides information about the interfacial chemistry of polymers may be necessary to gain further understanding. Electron spectroscopy for chemical analysis (ESCA) has been the principal technique that defines interfacial chemistries associated with adhesive behavior of materials. ESCA allows identification and quantitation of all elements with the exception of hydrogen (Ruse and Smith, 1990; Ratner and Castner, 1997). ESCA is an ideal technique to study surfaces of sealed films because of its surface sensitivity (1-10nm; Gerenser, 1993).

Lee (1994) studied surfaces of polyimide films using ESCA. Polar functional groups, such as hydroxy (OH), aldehyde (CHO), and carboxylic acid (COOH), have been identified on the surfaces of polyimide films. Reportedly, modifications of these functional groups were responsible for the adhesion strength differences of the films. Kawabe et al. (1999) employed ESCA to investigate surface modification of adhesive tapes on their adhesion behavior. Nitrogen plasma-treated tapes showed enhanced adhesion. They conclude that adhesion strength increased due to cross-linking reactions across the surfaces as determined by ESCA. As with many other biopolymer-based films, the sealing mechanism of whey protein-based films are not known, and no study has ever employed ESCA to investigate seal properties of biopolymer-based films.

The principle of the ESCA technique involves detecting the electrons emitted from specimen's atoms by absorption of photons. Another acronym simultaneously used is X-ray photoelectron spectroscopy (XPS), since X-ray is the source of the photons. X-ray excitation is used to yield emission of electrons form the core levels. This involves ionization of core electrons with bonding energies smaller than 1000 eV (Cayless, 1991). A schematic illustration of photoelectron emission is shown in Figure 1.7. A sample is irradiated with X-ray of known energy, $h\nu$, and electrons of binding energy, E_b, are ejected, where $E_b < h\nu$. These electrons have a kinetic energy, E_k which can be measured in the spectrometer.

$$E_k = h\nu - E_b - \Phi_{sp} \qquad (6)$$

where Φ_{sp} is the work function of the spectrometer and a constant. Thus, by measuring the kinetic energy of the photoelectrons, binding energy of the electrons can be obtained. An ESCA spectrum can be generated by plotting the measured photoelectron intensity as a function of binding energy. The binding energy is characteristic for each element.

$$E_b = h\nu - E_k \qquad (7)$$

A schematic diagram of the main components of an ESCA instrument is shown in Figure 1.8. The main components of the system, the X-ray source, sample stage, lens, analyzer, and detector are enclosed in an ultra high vacuum chamber (Kibel, 1991; Olefjord, 1997).

1.4.5 Moisture sorption properties

A moisture sorption isotherm (MSI) is a plot of correspondent equilibrium moisture content (EMC) of a material with its water activity (a_w) at constant temperature. Adsorption isotherms are obtained by addition of water to previously dried samples.

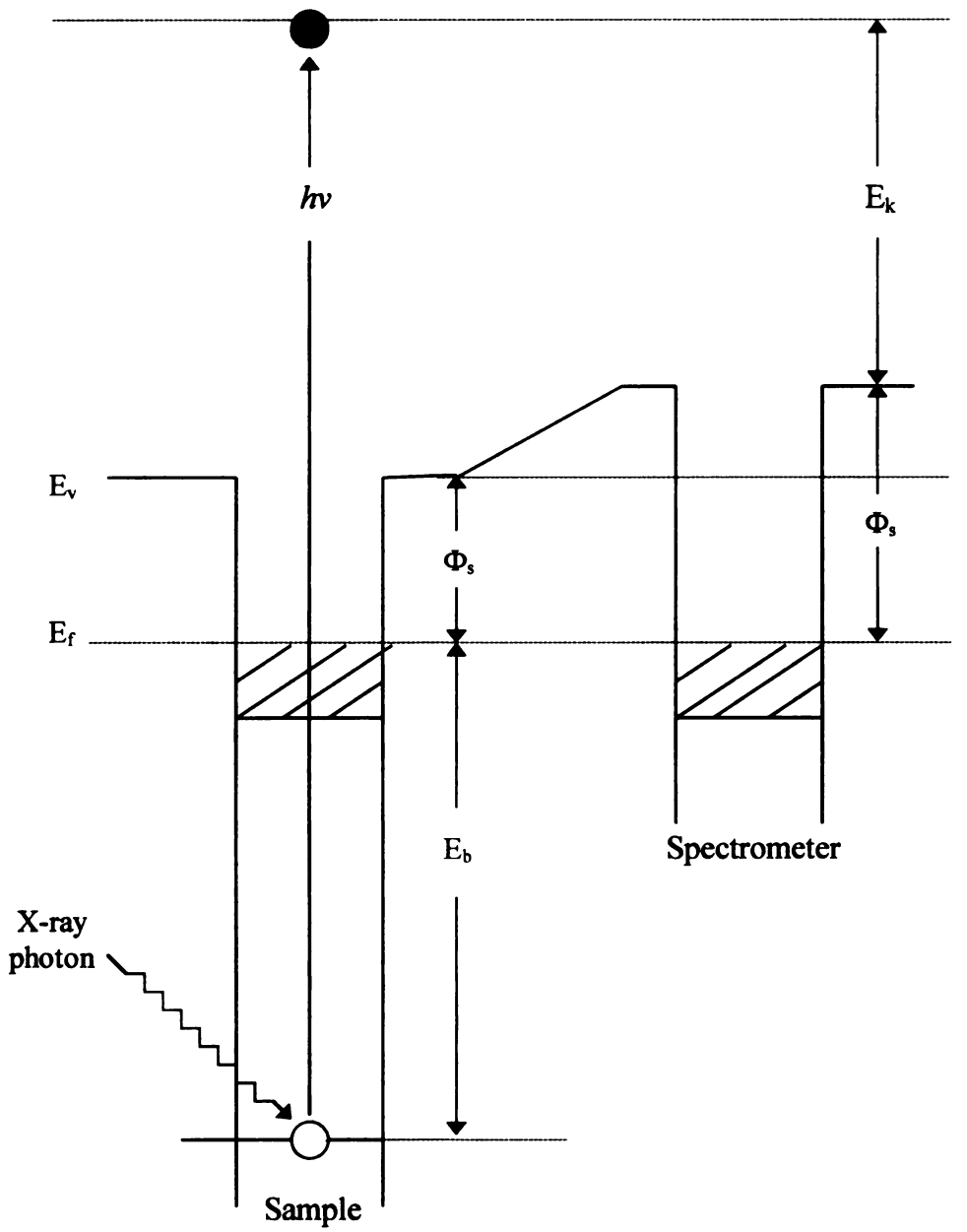


Figure 1.7. Schematic diagram of the photo emission process (Kibel, 1991).
$h\nu$ = energy of X-ray; E_b = binding energy of electrons; E_k = kinetic energy of electrons;
E_f = fermi level, the highest occupied energy level of both sample and spectrometer;
E_v = vacuum level; Φ_s = work formation of sample; Φ_{sp} = work formation of spectrometer

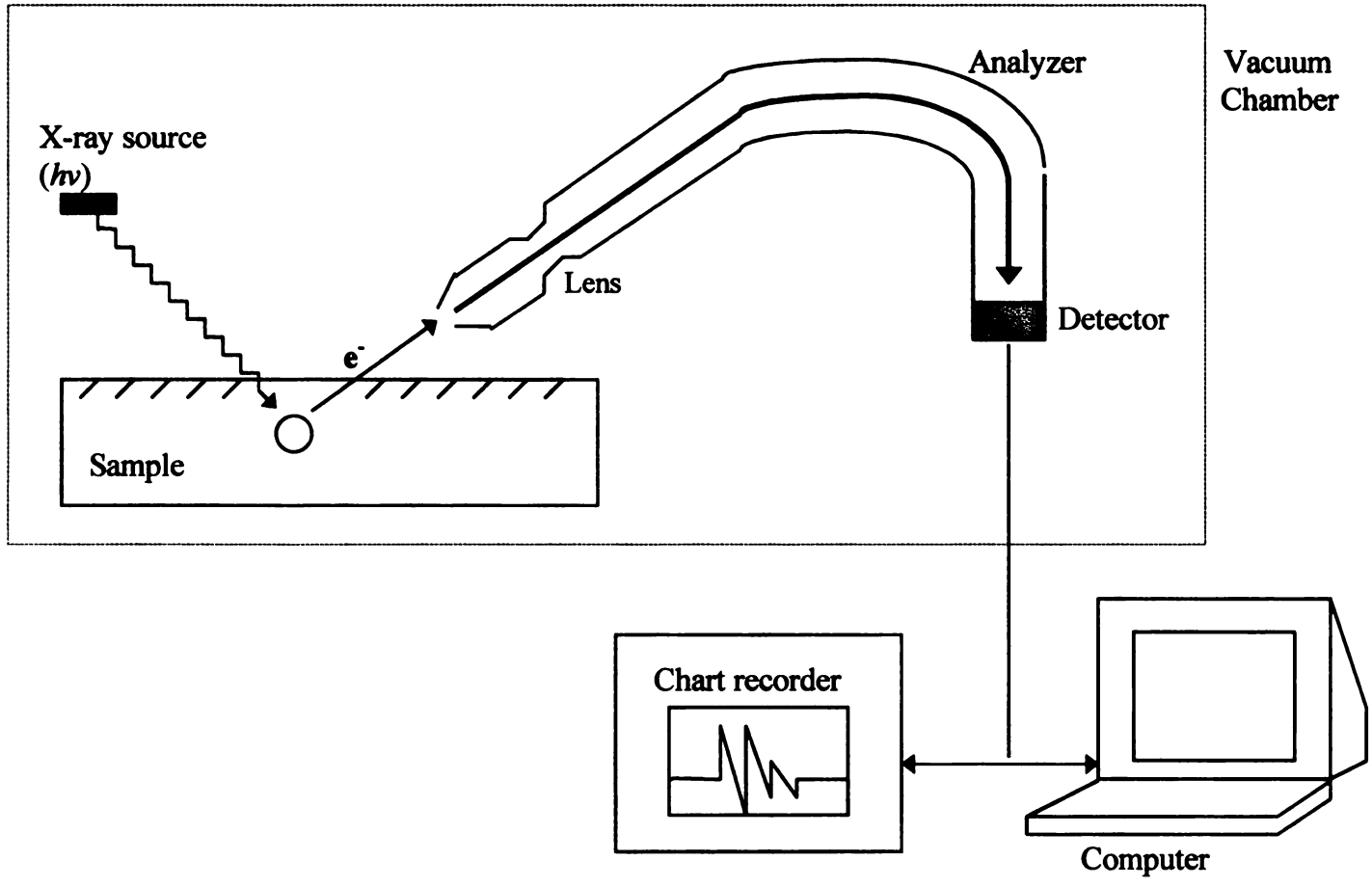


Figure 1.8. Schematic diagram of the electron spectroscopy for surface chemical analysis instrument (Ratner and Castner, 1997).

Desorption isotherms are prepared by removal of water from samples. Although these two sorption isotherms will not necessarily be superimposed, an adsorption isotherm is of particular interest when considering MSI of edible films. Moisture adsorption significantly affects stability, quality attributes, acceptability, and packaging and storage requirements of food products (Kinsella, 1984; Fennema, 1985).

Examining moisture sorption properties of edible films is useful in predicting water vapor transfer through films under various relative humidity (RH) conditions. The MSI also can be used to estimate properties of films at different environmental conditions for their appropriate application and to predict potential problems like reduced storage stability (McHugh and Krochta, 1994a; Jangchud and Chinnan, 1999). One of the simplest methods for obtaining a sorption isotherm is suggested by Labuza (1984). Films are placed in hermetically sealed containers at controlled RH and temperature. To obtain specific RH inside of containers, saturated salt solutions must be prepared by ASTM E-104 "*Standard practice for maintaining constant relative humidity*" (ASTM, 1997). After equilibrium is reached, moisture content of film samples are measured. This method has been employed in several studies to examine moisture sorption properties of protein-based edible films. Gontard et al. (1993) showed the difference between sorption and desorption isotherms of wheat gluten-based edible films. Lim et al. (1998) investigated MSI of egg-white protein films plasticized with varying plasticizer levels. Reportedly, the higher plasticizer levels induced higher EMC in the films resulted in films with increased hydrophilicity. Lai and Padua (1998) studied MSI of zein-based films. They reported that the MSI of zein-films were not typical sigmoidal curves as in the case of foods. Instead, it

resembled the MSI of crystalline sugar: very little moisture gain at $a_w < 0.7$, and exponential moisture sorption increase at $a_w > 0.7$.

There are number of food isotherm equations available in the literature. The four most commonly used isotherm equations are, Guggenheim-Anderson-de Boer (GAB), Braunauer-Emmett-Teller (BET), Smith, and Henderson equation (Jangchud and Chinnan, 1999). Among them, GAB equation is considered the most appropriate equation for modeling the moisture sorption characteristics of the films (Lim et al., 1999; Coupland et al., 2000). GAB equation is given as:

$$MC = W_m k C a_w / [(1 - k a_w)(1 - k a_w + c k a_w)] \quad (8)$$

where, MC = moisture content of the samples on a dry basis (g / 100 g dry solids)

W_m = monolayer moisture content

k, C = factors correcting differences in enthalpy of free and monolayer water compared to that of multilayer water, respectively.

a_w = water activity

In order to estimate parameters, the equation was transformed into a quadratic form as :

$$a_w / EMC = \alpha a_w^2 + \beta a_w + \gamma \quad (9)$$

where,

$$\alpha = k / W_m [1 / C - 1] \quad (10)$$

$$\beta = 1 / W_m [1 - 2 / C] \quad (11)$$

$$\gamma = 1 / W_m [C \times k] \quad (12)$$

CHAPTER 2

DEVELOPMENT OF WHEY PROTEIN/ LIPID EMULSION EDIBLE FILMS AND DETERMINATION OF THEIR MICROSTRUCTURE, BARRIER AND MECHANICAL PROPERTIES.

2.1 ABSTRACT

Methodologies were developed to produce edible films from whey protein isolate (WPI) and concentrate (WPC), and a film-forming procedure was optimized. Lipids, butter fat (BF) and candelilla wax (CW), were added into film-forming solutions to produce whey protein/lipid emulsion edible films. Effects of protein and lipid types and concentrations on moisture and oxygen barrier properties of whey protein/lipid emulsion films were investigated. Cross-sections and top surfaces of the films were examined using scanning electron microscopy. Significant reductions in water vapor and oxygen permeabilities of the films could be achieved upon addition of BF and CW. Tensile strength and percent elongation of whey protein/lipid emulsion films were affected primarily by protein type with some influenced by the lipid. Differences in the microstructures of the films accounted for the differences in their barrier and mechanical properties.

2.2 INTRODUCTION

Poor performance of whey protein-based films as moisture barriers is one of the main limitations for their application as food packaging materials. This is due to the hydrophilic nature of the proteins. Lipid films, on the other hand, act as good moisture barriers but have poor oxygen barrier properties and mechanical strength. The advantages of each can be achieved by combining whey proteins with lipids to produce composite films with improved properties (McHugh, 1996).

Protein/lipid composite films can be obtained using emulsions or layering technique. Generally, two or more layered films show better mechanical and barrier properties than emulsion films. However, the layering technique requires a complex and time-consuming film-manufacturing process, and the films tend to delaminate due to high surface energy on the interface (Shellammer and Krochta, 1997). Emulsion films, however, require only one-step operation, and the emulsifying property of whey proteins makes them good candidates to be used in emulsion films.

Various lipids have been examined for their effectiveness on properties of whey protein/lipid emulsion films. Fatty acids, waxes and butter fat (or milk fat) have been used in whey protein/lipid emulsion films and their properties have been reported (McHugh and Krochta, 1994c; 1994e; Shellhammer and Krochta, 1997). McHugh and Krochta (1994c; 1994e) investigated effects of fatty acids and beeswax on water vapor permeability (WVP) of whey protein/lipid emulsion films. They reported that increasing fatty acids or beeswax concentrations reduced the WVP of whey protein/lipid emulsion films. Shellhammer and Krochta (1997) studied mechanical properties of whey protein/lipid emulsion films when high-melting milk fat fraction, beeswax, carnauba wax and candelilla wax were

incorporated into film-forming solutions. The high-melting milk fat fraction and beeswax-added films showed relatively lower WVP compared to those of candelilla wax- or carnauba wax-added films (40% of the film dry wt.). Lipid type and concentration affected mechanical properties of the films. Overall, increasing the lipid concentration reduced the tensile strength (TS) of the films. Wax-added films showed no difference in percent elongation (%E) upon increasing lipid levels. However, there was a significant increase in %E of the films as the high-melting milk fat fraction level was increased.

Previous studies on whey protein/lipid emulsion films have been directed at their moisture barrier or mechanical properties, however, they have been limited. Fewer studies have been conducted on oxygen permeability (OP) of whey protein/lipid emulsion films. The main objective of this study was to evaluate the effect of lipids on moisture and oxygen barrier, and mechanical properties of whey protein/lipid emulsion films. Two types of whey proteins, whey protein isolate and whey protein concentrate, were selected to determine their feasibility and effectiveness as film-forming agents. Lipids (butter fat and candelilla wax) were added into film-forming solutions in various concentrations. Film formation procedure and formula ratio were optimized for whey protein/lipid emulsion films. The effect of protein and lipid type and concentration on barrier (WVP and OP) and mechanical properties (TS and %E) was investigated. Microstructures of the films were determined to illustrate the relationship between microstructures, and barrier and mechanical properties of the films.

2.3 MATERIALS AND METHODS

2.3.1 Materials

Whey protein isolate (ALACEN 891) and whey protein concentrate (ALACEN 879) were obtained from New Zealand Milk Products (North America) Inc., (Santa Rosa, CA). Table 2.1 shows the composition of the whey proteins used in the production of edible films in this study. Their protein contents were confirmed using AOAC 930.29 Kjeldahl method (AOAC, 1990). Candelilla wax was purchased from Strahl and Pitsch Inc. (West Babylon, NY), and unsalted butter fat was obtained from Land O'Lakes Inc. (Arden Hills, MN). D-Sorbitol was purchased from Sigma Chemical Co.(St. Louis, MO), and NaOH was purchased from Mallinckrodt Specialty Chemical Co. (Paris, KY).

2.3.2 Film preparation

Figure 2.1 shows the schematic diagram of the film-forming process. Edible film-forming solutions were prepared by mixing whey protein isolate (WPI) or whey protein concentrate (WPC; 5%, w/v) with sorbitol, 2N NaOH (to adjust pH), and distilled H_2O to a final volume of 100ml. The pH of the solutions were adjusted to 8. These solutions were heated to a final temperature of 90 ± 2 °C for 15 minutes while being stirred continuously (Model 4820-4 "Magna-4" magnetic stirrer and hot plate, Cole-Parmer, Chicago, IL). Butter fat (BF; 0.1 or 0.2%, w/v) or candelilla wax (CW; 0.2, 0.4, or 0.8%, w/v) was added during the heat treatment and allowed to melt into the protein solutions. BF used in this study contained 15.8% of moisture (w/w), determined in drying oven (Precision Scientific model 524, Chicago, IL) at 100 ± 2°C for 3 h (AOAC, 1990). BF

Table 2.1. Composition of whey proteins used[1].

Components	Whey protein isolate ALACEN 891	Whey protein concentrate ALACEN 879
Protein (N x 6.38) %	90.0	80.0
Ash %	2.0	4.3
Moisture %	3.8	4.2
Fat %	0.2	5.2
Lactose %	4.5	5.2
pH[2]	6.5	7.0

[1] Values based on specifications provided by New Zealand Milk Products (N. America) Inc.
[2] 5% at 20°C

Mix film forming components

WPI or WPC, sorbitol and distilled H_2O

⇓

Adjust solution pH to 8 with 2N-NaOH

⇓

Heat treatment (90°C, 15 min, stirring)

Add lipids, BF or CW, during heat treatment

⇓

Homogenize (2 min)

⇓

Equilibrate (2 hours at 23 ± 2°C)

⇓

Filter through cheese cloth

⇓

Vacuum for de-gassing

⇓

Cast solution onto teflon coated pans using pipette

⇓

Dry (23 ± 2°C, 30 ± 5% RH, 18 ± 3hr)

⇓

Peel films from casting surface

⇓

Store samples at ambient condition before testing (23 ± 2°C, 50 ± 5 % RH)

Figure 2.1. Schematic diagram of film-forming process.

was chosen due to its compatibility with whey powders; also it has been reported to be an effective material in reducing WVP of protein-based films (Shellhammer and Krochta, 1997). CW was selected after the preliminary test. It showed no waxy texture like beeswax and provided better WVP than that films containing carnauba wax.

Next the solutions were homogenized for 2 minutes using the Polytron PT 10/35 homogenizer with a PTA 20 TS generator (homogenizing head) and a PCU 11 power control unit at setting 5 (Tekmar Co., Cincinnati, OH). The solutions were allowed to equilibrate at 23 ± 2°C for 2 h. The mixtures were filtered through two layers of cheesecloth, and were de-gassed using a hydrometric vacuum system. Casting was performed by pipetting the solutions on to teflon coated pans, 18.5 cm in diameter and placed on a leveled surface. Drying was carried out at 23 ± 2°C and 30 ± 5% RH for 18 ± 3 h. Films were peeled from the casting surface and stored at 23 ± 2°C and 50 ± 5% RH until tested. Table 2.2 shows the composition of the films produced.

2.3.3 Determination of thickness

A TMI model 549 M micrometer (Testing Machines, Inc. Amityville, NY) was used to measure film thickness. For determining barrier and mechanical properties, measurements were taken at five different location and the mean value was used for calculations. Barrier and mechanical testing were done on film samples of 0.140 ± 0.019 mm (5.5 mils).

Table 2.2. Composition of whey protein/lipid emulsion edible films produced.

Treatments [1]	% w/v of aqueous solution			% w/w of protein	
	Protein [2]	Sorbitol	Lipid	Sorbitol	Lipid
IS	5	5.0	0	100	0
IS-BF2%	5	4.9	BF = 0.1	98	BF = 2
IS-BF4%	5	4.8	BF = 0.2	96	BF = 4
IS-CW4%	5	4.8	CW = 0.2	96	CW = 4
IS-CW8%	5	4.6	CW = 0.4	92	CW = 8
IS-CW16%	5	4.2	CW = 0.8	84	CW = 16
CS	5	5.0	0	100	0
CS-BF2%	5	4.9	BF = 0.1	98	BF = 2
CS-BF4%	5	4.8	BF = 0.2	96	BF = 4
CS-CW4%	5	4.8	CW = 0.2	96	CW = 4
CS-CW8%	5	4.6	CW = 0.4	92	CW = 8
CS-CW16%	5	4.2	CW = 0.8	84	CW = 16

[1] I = whey protein isolate; S = sorbitol; BF = butter fat; CW = candelilla wax; C = whey protein concentrate.

[2] whey protein isolate or whey protein concentrate

2.3.4 Water vapor permeability

Water vapor permeability (WVP) was tested using the Permatran-W3/31 (MoCon, Inc., Minneapolis, MN), according to ASTM F-1249, "*Standard test method for water vapor transmission rate through plastic film and sheeting using a modulated infrared sensor*" (ASTM, 1997). The testing surface area of the whey protein-based film samples were reduced from 50cm^2 to 5cm^2 with the use of a aluminum foil backing to stay within sensor range.

Two different relative humidities (50 ± 3 and 80 ± 3% RH) were used while temperature was kept constant at 25 ± 0.5 °C. Samples were conditioned for 5 h at the RH condition which testing was conducted. Permeability was determined once a steady state was reached. Steady state occurs when permeation of the gas or vapor is constant. Calibration of the system was executed with 0.025mm (1mil) thick standard polyester (PET) film. The WVP was reported in g · mm/m^2 · day · kPa, for all films by converting the water vapor transmission rate values (WVTR) obtained from the instrument (g/m^2 · day), with the use of the appropriate conversion units. WVP was calculated by the following equation:

$$WVP = WVTR \times d / \Delta p \qquad (13)$$

WVTR = water vapor transmission rate of the test film

d = thickness of film

Δp = pressure differential acting on film

2.3.5 Oxygen permeability

Oxygen permeability (OP) test was conducted according to ASTM D-3985, "*Standard test method for oxygen gas transmission rate through plastic film and sheeting using a coulometric sensor*" (ASTM, 1997), using the Oxtran 200 (MoCon, Inc., Minneapolis, MN). Films were cut into 11 x 11cm square samples and masked with an aluminum foil mask, making the area to be tested $5cm^2$, and placed in the $50cm^2$ cell of the tester. The tester was equipped with an Endocal Temperature Control Bath model RTE 100 (Neslab Instrument Inc., Newington, USA.), and conformed with standard ASTM D-3985 method.

The test was run at 23 ± 0.5°C and 0% RH using nitrogen (containing 2% hydrogen) as the carrier gas, and air (21% oxygen) as the test gas. All samples were conditioned for 5 h under the same conditions prior to testing. The flow was measured until steady state was reached. Calibration of the system was executed with 0.025mm (1mil) standard PET film. The OP was reported in $cm^3 \cdot \mu m/m^2 \cdot day \cdot kPa$ for all films by converting the oxygen transmission rate values (OTR) obtained from the instrument ($cm^3/m^2 \cdot day$) with the use of the appropriate conversion units and the following equation:

$$P = (OTR \times d) / \Delta p \qquad (14)$$

d = thickness of film

Δp = pressure differential acting on the film

2.3.6 Mechanical properties

Films samples were cut into strips of 2.54cm wide using a Precision Sample Cutter (Thawing Albert Instrument Co., Philadelphia, PA). All films were conditioned for 48 h at

the same test conditions prior to testing. Tensile strength (TS) and percent elongation (%E) was determined according to ASTM D-882, "*Standard test method for tensile properties of thin plastic sheeting*" (ASTM, 1997). Test were run using the Instron Universal Testing Machine Model 2401 (Instron Corp., Canton, MA), at 23 ± 2°C and 50 ± 5% RH. A 1 kN static load cell and cross head speed of 20 in/min was used. Testing sample size was 2.54cm x 8cm. The MPa value of TS was calculated from the following equation:

$$TS = \text{load} / \text{area} \quad (15)$$

load = peak force

area = sample width x sample thickness

%E was determined by the equation:

$$\%E = \Delta L / L_i \times 100 \text{ (expressed as a percentage)} \quad (16)$$

ΔL = change in length

L_i = original length of sample

2.3.7 Scanning Electron Microscopy

Microstructure of the films were determined using the Scanning Electron Microscope (SEM) JSM-6400V (Japan Electron Optics Laboratory, Tokyo, Japan; Appendix III). Cross-sections and surfaces of the films were examined. A film cross-section was obtained by cross cutting using a razor blade. Both samples were mounted on aluminum stubs with double-sided cellophane tape and carbon conductive paint. Each sample was ion sputtered with gold-palladium alloy (gold deposit: 10 nm) for 3 minutes with 20 mA current. Samples were observed with accelerating voltage of 15 kV. Two

different magnifications of 400x and 1,500x were used to examine cross-sections and surfaces of the films, respectively.

2.3.8 Statistical analysis

All experiments were replicated three times in a randomized block experiment. A new film forming solution and new set of films were prepared for each replicate. Statistical analysis were made using Sigma Stat 2.0 (Jandel Corp., San Rafael, CA) and appropriate comparisons were done using the Student-Newman-Keuls method for multiple comparisons.

2.4 RESULTS & DISCUSSION

Analysis of the whey protein isolate (WPI) and concentrate (WPC) powders showed them to contain 90.8 and 81.8% protein, respectively. Films produced from WPI and WPC with sorbitol as the plasticizer were clear, smooth, and flexible. CW added films showed slight opaqueness in both WPI and WPC films. Figure 2.2 shows representative films. CW could not be incorporated above 16% (dry basis, w/w of protein), because higher wax contents produced films that could not be peeled off from the casting surface. Above 2% (dry basis, w/w of protein) BF incorporated films had a greasy surface. Overall, increased lipid levels induced more translucent films. The thickness of the films averaged of 140 ± 19μm. Whey protein type and incorporation of lipids did not affect film thickness.

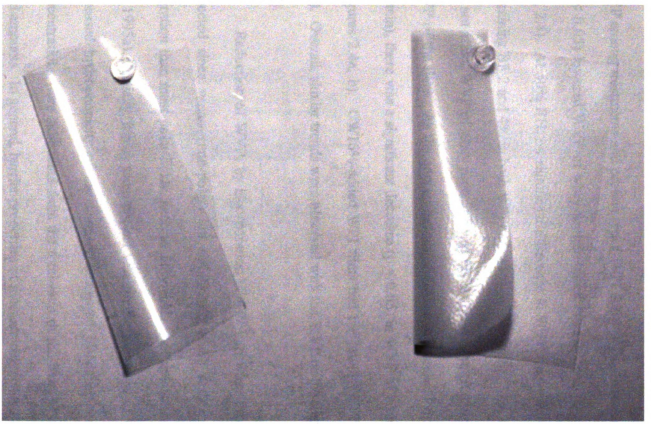


Figure 2.2. A representative whey protein isolate-based film (shown on left); whey protein isolate-based film containing candelilla wax (shown on right).

2.4.1 Water vapor permeability

Figures 2.3 and 2.4 show WVP of BF- and CW-added whey protein-based films tested at 50 and 80% RH, respectively. Table 2.3 shows the statistical comparison of WVP among treatments and protein types, WPI and WPC. Addition of lipids significantly ($p < 0.05$) lowered WVP of whey protein-based films at both 50 and 80% RH (Figures 2.3, 2.4). At 50% RH, no significant difference in WVP was observed between the films containing BF 2 and 4% in both WPI and WPC films (Figures 2.3a, b). Overall, BF levels did not affect WVP of whey protein-based film. CW was much more effective in lowering WVP of the films. As the concentrations of CW were increased from 4 to 16% (w/w of protein), there was a significant decrease ($p < 0.05$) in WVP of both WPI and WPC films (Figures 2.4a, b). CW16%-added WPI films had the lowest WVP at both RHs (Table 2.3). Overall, similar trends were observed with the WVP of the films at 80% RH (Figure 2.4).

Reduction of WVP, by incorporation of CW into whey protein-based films, was expected since waxes (non-polar) are significantly more resistant to moisture vapor migration than most polar-lipids, such as fatty acids and acylglycerol type lipids (Cuq et al., 1995a). Initially, it may seem unclear whether the reduction of WVP occurred due to increased hydrophobicity of the films or decreased plasticizer levels, since plasticizer concentrations were subtracted from the total ratio of the films to compensate for added lipid amounts. In general, barrier properties of films are negatively affected by plasticizers. Since plasticizers reduce the rigidity of film structure, diffusion of moisture vapor molecules through films become easier at higher plasticizer concentrations (Gennadios et al., 1993c). However, Chick and Ustunol (1998) reported that the protein/plasticizer ratio

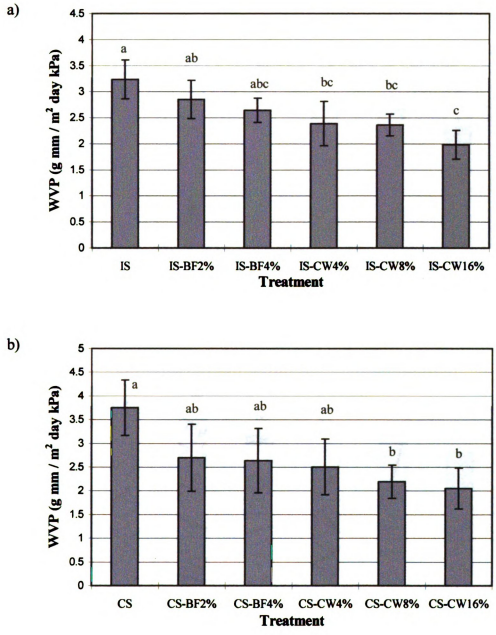


Figure 2.3. Water vapor permeability (WVP) of whey protein/lipid emulsion edible films (tested at 25°C and 50% RH).
(a) whey protein isolate films; (b) whey protein concentrate films
I = whey protein isolate; C = whey protein concentrate;
S = sorbitol; BF = butter fat; CW = candelilla wax

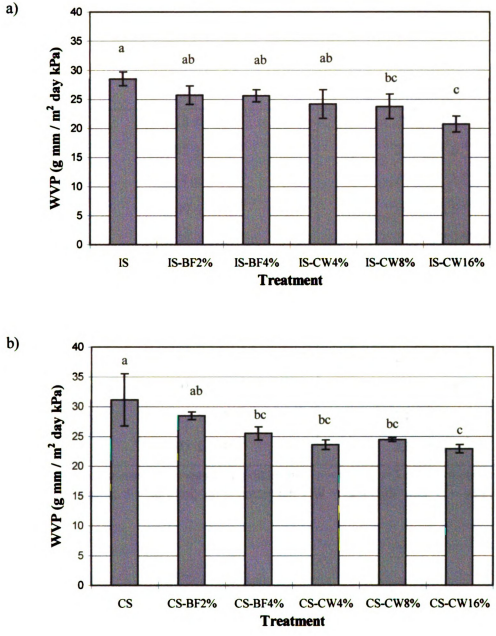


Figure 2.4. Water vapor permeability (WVP) of whey protein/lipid emulsion edible films (tested at 25°C and 80% RH).
(a) whey protein isolate films; (b) whey protein concentrate films
I = whey protein isolate; C = whey protein concentrate;
S = sorbitol; BF = butter fat; CW = candelilla wax

Table 2.3. Water vapor permeability (WVP) of whey protein/lipid emulsion edible films (at 25°C).

Treatment [1]	WVP (g. mm/ m^2. d. kPa)	
	50% RH	80% RH
IS	3.23 ± 0.37 [ab]	28.5 ± 1.2 [ab]
IS-BF2%	2.85 ± 0.36 [ab]	25.7 ± 1.6 [ab]
IS-BF4%	2.64 ± 0.23 [ab]	25.6 ± 1.1 [ab]
IS-CW4%	2.39 ± 0.42 [b]	24.1 ± 2.5 [ab]
IS-CW8%	2.36 ± 0.21 [b]	23.8 ± 2.1 [ab]
IS-CW16%	1.98 ± 0.27 [b]	20.7 ± 1.4 [b]
CS	3.75 ± 0.58 [a]	31.1 ± 4.4 [a]
CS-BF2%	2.69 ± 0.74 [ab]	28.4 ± 0.7 [ab]
CS-BF4%	2.63 ± 0.68 [ab]	25.4 ± 1.1 [ab]
CS-CW4%	2.50 ± 0.59 [ab]	23.5 ± 0.8 [ab]
CS-CW8%	2.19 ± 0.35 [b]	24.4 ± 0.3 [ab]
CS-CW16%	2.05 ± 0.43 [b]	22.9 ± 0.7 [ab]

[a-b] Different letters columnwise denote significant difference ($p < 0.05$), n=3 for all treatments.
[1] I = whey protein isolate; S = sorbitol; BF = butter fat; CW = candelilla wax; C = whey protein concentrate.

Table 2.4. Water vapor permeability (WVP) of various protein-based edible films and synthetic polymers.

Films [1]	WVP (g.mm/ m^2.d.kPa)	Test conditions (temp., RH)	References
WG : G (5:1)	8.3	30°C, 0/100%	Gontard et al (1994)
WG : AM : G (5:1.5:1)	10.5	30°C, 0/100%	Gontard et al (1994)
WG : SL : G (5:1.5:1)	9.0	30°C, 0/100%	Gontard et al (1994)
WG : PW : G (5:1.5:1)	6.4	30°C, 0/100%	Gontard et al (1994)
WG : CBW : G (5:1.5:1)	6.0	30°C, 0/100%	Gontard et al (1994)
WG : BW : G (5:1.5:1)	3.0	30°C, 0/100%	Gontard et al (1994)
SC (no plasticizer)	36.7	25°C, 0/81%	Avena-Bustillos and Krochta (1993)
SC : AM (4:1)	25.4	25°C, 0/88%	Avena-Bustillos and Krochta (1993)
SC : BW (4:1)	13.2	25°C, 0/88%	Avena-Bustillos and Krochta (1993)
LAC : G (1:1)	59.3	38°C, 0/90%	Chick and Ustunol (1998)
LAC : G (0.6:1)	54.9	38°C, 0/90%	Chick and Ustunol (1998)
LAC : S (1:1)	2.8	38°C, 0/50%	Chick (1998)
LAC:CBW : S (1.1:0.1:1)	2.9	38°C, 0/50%	Chick (1998)
LAC:CBW : S (1.4:0.4:1)	1.3	38°C, 0/50%	Chick (1998)
LAC:CW : S (1.1:0.1:1)	1.7	38°C, 0/50%	Chick (1998)
LAC:CW : S (1.4:0.4:1)	0.8	38°C, 0/50%	Chick (1998)
LAC:CW : S (1.1:0.1:1)	8.1	38°C, 0/70%	Chick (1998)
LAC:CW : S (1.4:0.4:1)	6.2	38°C, 0/70%	Chick (1998)
WPI : S (1.3:1)	52.0	25°C, 0/70%	McHugh and Krochta (1994c)
WPI : MA : S (1.3:1.8:1)	23.8	25°C, 0/70%	McHugh and Krochta (1994c)
WPI : PA : S (1.3:1.8:1)	19.2	25°C, 0/70%	McHugh and Krochta (1994c)
WPI : G (15:1)	45.4	25°C, 0/88%	Shellhammer and Krochta (1997)
WPI : CW : G (15:11:1)	30.9	25°C, 0/88%	Shellhammer and Krochta (1997)
WPI : MF : G (15:11:1)	21.9	25°C, 0/88%	Shellhammer and Krochta (1997)
WPI : BW : G (15:11:1)	10.8	25°C, 0/88%	Shellhammer and Krochta (1997)
Low density polyethylene	0.08	38°C, 90/0%	Smith (1986)
High density polyethylene	0.02	38°C, 90/0%	Smith (1986)
Cellophane	7.27	38°C, 90/0%	Taylor (1986)

[1] WG = wheat gluten; SC = sodium caseinate; LAC = lactic acid casein; WPI = whey protein isolate; G = glycerol; S = sorbitol; AM = acetylated monoacylglycerol; BW = beeswax; SL = soya lecithin; PW = parafin wax; CBW = carnauba wax; CW = candelilla wax; MA = myristic acid; PA = palmitic acid; MF = milkfat.

had no effect ($p > 0.05$) on WVP of lactic acid casein-based films (Table 2.4). Apparently, a protein:plasticizer ratio change from 1:1 to 0.6:1 was not significant enough to affect WVP of the films. In the present study, the change in the protein:plasticizer ratio was less than that reported by Chick and Ustunol (1998), and also there were differences in WVP of BF4%- and CW4%-added films with the same amount of plasticizer (Tables 2.2, 2.3). This leads me to believe that the decrease in WVP is due to the lipids incorporated rather than due to the reduction in the amount of plasticizer in the films.

The protein type, WPI and WPC, did not affect WVP of whey protein-based films (Table 2.3). With an increase in RH from 50 to 80%, the WVP of all treatments increased ~10 times. Similar results were observed by Chick (1998). Reportedly, RH affected WVP of CW-added lactic casein acid-based films exponentially. According to Gennadios et al. (1993c), protein-based films are prone to swell in high RH environments because of high water sorption rates. This leads to a diffusivity increase of water molecules and results in increased WVP of protein-based films at high RH.

The WVP results of this study were comparable to those of most protein/lipid emulsion edible films (Table 2.4). In particular, when comparing WVP data in this present study to the results from the study by Shellhammer and Krochta (1997), the WVP data from this study showed a greater effectiveness of lipid addition on lowering WVP of whey protein-based films. They reported WVP of 30.9 and 21.9 $g.mm/m^2.day.kPa$ for CW and milk fat-added WPI films, respectively (Table 2.4). Approximately 70% (w/w of protein) lipid were incorporated in the films to obtain their results, while only 4% BF and 16% CW (w/w of protein) were required to achieve similar WVP values in our study, 24.1 and 20.7 $g.mm/m^2.day.kPa$, respectively (Table 2.3). However, caution needs to be taken in

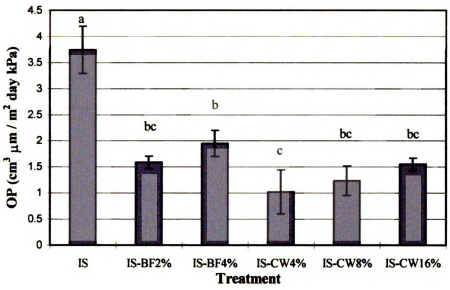
comparing data obtained from different studies by different investigators, since the material resources, film preparation, procedures, equipment and testing conditions were slightly different in each of the studies.

2.4.2 Oxygen permeability

Figure 2.5 shows OP of whey protein/lipid emulsion films. A statistical comparison of OP between WPI and WPC films is shown in Table 2.5. A significant decrease ($p < 0.05$) was observed in OP with the addition of lipids, BF and CW, to WPI and WPC films (Figures 2.5a, b). All treatments with lipids showed lower OP compared to those with no lipid added. Similar results were observed by Gennadios et al. (1993c). Incorporation of a lipid (acetylated monoacylglycerol) decreased OP of wheat gluten-based films from 3.9 to 2.7 $cm^3 \cdot \mu m/m^2 \cdot d \cdot kPa$ (Table 2.6).

In comparing effect of lipid types (BF and CW) on OP of WPI films, CW was more effective ($p < 0.05$) than BF in reducing OP of the films (Figure 2.5a). This comparison was made within the same lipid level of 4% (w/w of protein). A similar trend was observed in WPC films (Figure 2.5b). In general, waxes show good barrier properties since waxes present a tight orthorhombic crystalline arrangement that is perpendicular to the direction of the gas flow (Donhowe and Fennema, 1993). According to Callegarin et al. (1997), in addition to the nature of film-forming substances, surface morphology and homogeneity of the film matrix are also important factors that affect barrier properties of films. Detailed discussion with regards to surface morphology and microstructure will be provided in section 2.4.5 including Scanning Electron Micrographs.

a)



b)

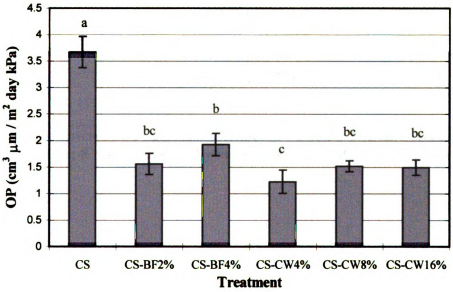


Figure 2.5. Oxygen permeability (OP) of whey protein/lipid emulsion edible films (tested at 23°C and 0% RH).
(a) whey protein isolate films; (b) whey protein concentrate films
I = whey protein isolate; C = whey protein concentrate;
S = sorbitol; BF = butter fat; CW = candelilla wax

Table 2.5. Oxygen permeability (OP) of whey protein/lipid emulsion edible films (23°C, 0% RH).

Treatment [1]	OP (cm^3. μm/ m^2. d. kPa)
IS	3.74 ± 0.45^{a}
IS-BF2%	1.59 ± 0.12^{bc}
IS-BF4%	1.95 ± 0.25^{b}
IS-CW4%	1.02 ± 0.42^{c}
IS-CW8%	1.23 ± 0.28^{c}
IS-CW16%	1.55 ± 0.12^{bc}
CS	3.67 ± 0.29^{a}
CS-BF2%	1.55 ± 0.20^{bc}
CS-BF4%	1.93 ± 0.21^{b}
CS-CW4%	1.22 ± 0.22^{c}
CS-CW8%	1.52 ± 0.10^{bc}
CS-CW16%	1.49 ± 0.15^{bc}

[a-c] Different letters columnwise denote significant difference ($p < 0.05$), n=3 for all treatments.
[1] I = whey protein isolate; S = sorbitol; BF = butter fat; CW = candelilla wax; C = whey protein concentrate.

Table 2.6. Oxygen permeability (OP) of various protein-based edible films and synthetic polymers.

Films [1]	OP ($cm^3.\mu m/m^2.d.kPa$)	Test conditions (temp, RH)	References
WG : G (2.5:1)	3.9	23°C, 0%	Gennadios et al. (1993c)
WG : AM : G (2.5:0.2:1)	2.7	23°C, 0%	Gennadios et al. (1993c)
LAC : S (1:1)	0.4	23°C, 0%	Chick (1998)
LAC: CBW : S (1.1:0.1:1)	0.3	23°C, 0%	Chick (1998)
LAC: CBW : S (1.4:0.4:1)	0.5	23°C, 0%	Chick (1998)
LAC: CW : S (1.1:0.1:1)	0.3	23°C, 0%	Chick (1998)
LAC: CW : S (1.4:0.4:1)	0.6	23°C, 0%	Chick (1998)
WPI : S (3.5:1)	1.5	23°C, 46%	McHugh (1996)
WPI : BW : S (3.5:1.8:1)	8.6	23°C, 46%	McHugh (1996)
WPI : S (3.5:1)	42.3	23°C, 70%	McHugh (1996)
WPI : BW : S (3.5:1.8:1)	101.1	23°C, 70%	McHugh (1996)
Low density polyethylene	1865	23°C, 50%	Salame (1986)
High density polyethylene	427	23°C, 50%	Salame (1986)
Cellophane	0.7	23°C, 0%	Salame (1986)

[1] WG = wheat gluten; LAC = lactic acid casein; WPI = whey protein isolate; G = glycerol; S = sorbitol; AM = acetylated monoacylglycerol; CBW = carnauba wax; CW = candelilla wax; BW = beeswax.

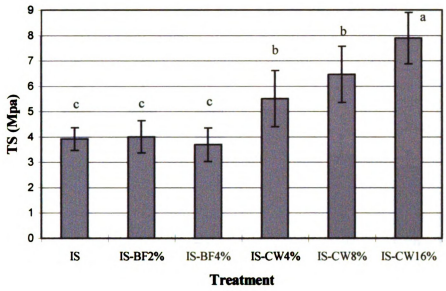
Whey protein type, WPI and WPC, did not affect OP of the films (Table 2.5). The CW4%-added WPI film had the lowest OP of 1.02 $cm^3 \cdot \mu m/m^2 \cdot d \cdot kPa$. This was approximately a 70% reduction compared to OP of the WPI film with no lipid added, 3.74 $cm^3 \cdot \mu m/m^2 \cdot d \cdot kPa$. Further addition of CW (8 and 16%, w/w of protein), however, increased OP. The oxygen barrier properties of whey protein/lipid emulsion films in this study were comparable to those of acetylated monoacylglycerol-added wheat gluten-based films, carnauba wax-added lactic acid casein-based films, and cellophane (Table 2.6). However, it is important that the OP of films are compared between films tested under the same testing conditions. RH conditions in particular have an exponential effect on OP of the protein-based films (Mujica-Paz and Gontard, 1997).

Whey protein-based films make excellent oxygen barriers because of their highly polar nature (Chen, 1995). Highly polar polymers like proteins exhibit high degrees of hydrogen bonding, which creates limited polymer chain motion, resulting in low gas permeability (McHugh and Krochta, 1994a). Thus, OP of protein-based films is often lower than that of common synthetic films such as LDPE and HDPE. For example, OP of WPI-based film was 500 times lower than that of LDPE, 3.74 compared to 1865 $cm^3 \cdot \mu m/m^2 \cdot d \cdot kPa$ (Tables 2.5, 2.6).

2.4.3 Tensile strength and percent elongation

Figures 2.6 and 2.7 show TS and %E of whey protein/lipid emulsion films. Table 2.7 shows the statistical comparison of TS and %E among protein types. With WPI films, addition of BF had no significant effect on TS and %E of the films. On the other hand, increasing CW levels increased TS of WPI films while a decline in %E was observed ($p <$

a)



b)

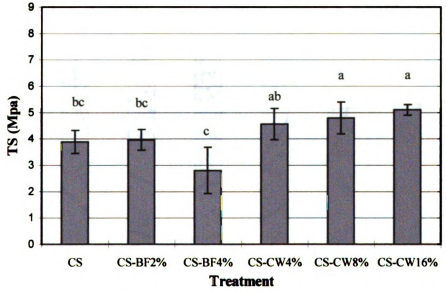


Figure 2.6. Tensile strength (TS) of whey protein/lipid emulsion edible films (tested at 23°C and 50% RH).
(a) whey protein isolate films; (b) whey protein concentrate films
I = whey protein isolate; C = whey protein concentrate;
S = sorbitol; BF = butter fat; CW = candelilla wax

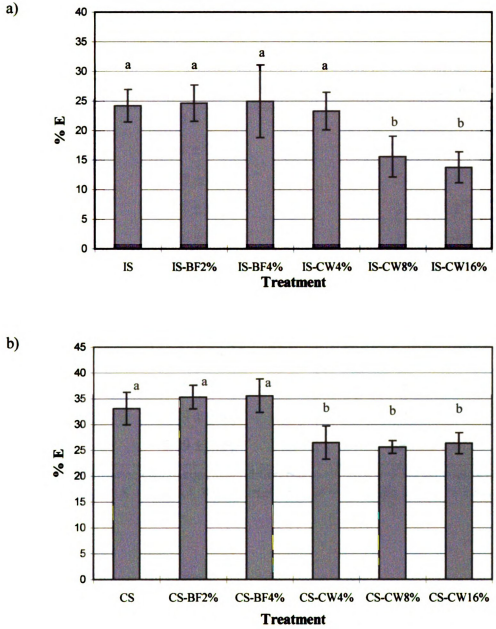


Figure 2.7. Percent elongation (%E) of whey protein/lipid emulsion edible films (tested at 23°C and 50% RH).
(a) whey protein isolate films; (b) whey protein concentrate films
I = whey protein isolate; C = whey protein concentrate;
S = sorbitol; BF = butter fat; CW = candelilla wax

Table 2.7. Mechanical properties of whey protein/lipid emulsion edible films (23°C, 50% RH).

Treatment [1]	Tensile strength (MPa)	Percent elongation (%)
IS	3.92 ± 0.45 [de]	24.2 ± 2.8 [b]
IS-BF2%	4.01 ± 0.64 [de]	24.6 ± 3.1 [b]
IS-BF4%	3.69 ± 0.66 [e]	24.9 ± 6.1 [b]
IS-CW4%	5.51 ± 1.11 [bc]	23.3 ± 3.2 [b]
IS-CW8%	6.46 ± 1.10 [b]	15.6 ± 3.4 [c]
IS-CW16%	7.89 ± 1.01 [a]	13.7 ± 2.6 [c]
CS	3.88 ± 0.43 [de]	33.1 ± 3.1 [a]
CS-BF2%	3.96 ± 0.39 [de]	35.3 ± 2.3 [a]
CS-BF4%	2.80 ± 0.88 [f]	35.6 ± 3.2 [a]
CS-CW4%	4.56 ± 0.59 [cde]	26.5 ± 3.2 [b]
CS-CW8%	4.79 ± 0.60 [cd]	25.6 ± 1.3 [b]
CS-CW16%	5.09 ± 0.20 [c]	26.3 ± 2.0 [b]

[a-f] Different letters columnwise denote significant difference ($p < 0.05$), n=3 for all treatments.
[1] I = whey protein isolate; S = sorbitol; BF = butter fat; CW = candelilla wax; C = whey protein concentrate.

Table 2.8. Mechanical properties of various protein-based edible films and synthetic polymers.

Films [1]	Tensile strength (MPa)	Percent elongation (%)	References
CZ : PA (1:0.5)	5.2	0.5	Lai et al. (1997)
CZ : PA (1:0.75)	14.6	1.0	Lai et al. (1997)
CZ : PA (1:1)	11.6	0.8	Lai et al. (1997)
CZ : OA (1:0.5)	9.4	5.9	Santosa and Padua (1999)
CZ : OA (1:0.75)	4.2	46.9	Santosa and Padua (1999)
CZ : OA (1:1)	2.2	7.5	Santosa and Padua (1999)
SPI : G (2:1)	6.3	112	Rhim et al. (1999a)
SPI : LA : G (2:0.6:1)	4.2	35.0	Rhim et al. (1999a)
SPI : PA : G (2:0.6:1)	4.8	20.0	Rhim et al. (1999a)
LAC : S (1:1)	6.2	156.0	Chick (1998)
LAC : CW : S (1.4:0.4:1)	8.3	37	Chick (1998)
WPI : G (2:1)	5.9	22.7	Banerjee and Chen (1995)
WPC : G (2:1)	3.4	20.8	Banerjee and Chen (1995)
WPI : AM : G (2:1:1)	3.2	10.8	Banerjee and Chen (1995)
WPC : AM : G (2:1:1)	1.1	13.6	Banerjee and Chen (1995)
Low density polyethylene	8.6 - 17	500	Briston (1988)
High density polyethylene	17 - 35	300	Briston (1988)
Cellophane	48 - 110	15 - 25	Briston (1988)

[1]CZ = corn zein; SPI = soy protein isolate; LAC = lactic acid casein; WPI = whey protein isolate; WPC = whey protein concentrate; G = glycerol; S = sorbitol; PA = palmitic acid; OA = oleic acid; LA = lauric aicd; CW = candelilla wax; AM = acetylated monoacylglycerol.

0.05; Figures 2.6a, 2.7a). Similar trends were observed with TS and %E of WPC films, except that the addition of BF decreased TS of WPC films (Figures 2.6b, 2.7b). The effectiveness of CW on TS and %E of lactic acid casein-based films was studied by Chick (1998). Similar to the results from my study, addition of CW significantly increased TS and decreased %E of lactic acid casein-based films. Reportedly, CW induced rigidity of whey protein/lipid emulsion films, which resulted in increased TS and decreased %E upon raised CW levels.

Unlike CW-added film, BF-added films exhibited somewhat different characteristics in terms of mechanical properties. Increased BF level did not increase TS values, while %E values remained the same compared to films without lipids incorporated. According to Callegarin et al. (1997), acylglycerol type lipids may increase flexibility of the films by weakening the intermolecular forces between adjacent polymer chains. BF contains 97-98% triacylglycerols (Sax and Lewis, 1987). Banerjee and Chen (1995) reported addition of acetylated monoacylglycerols reduced TS of whey protein-based films (Table 2.8).

In Table 2.7, WPI films showed higher ($p < 0.05$) TS values while WPC films had higher ($p < 0.05$) %E at all treatments. The highest TS of 7.89 MPa was observed with CW16%-added WPI films, and BF4%-added WPC films had the highest %E of 35.6%. Banerjee and Chen (1995) also reported WPI films to have higher TS than WPC films, 5.94 and 3.36 MPa, respectively. They suggested a lower protein concentration was responsible for the weaker films with WPC. The TS and %E values of my films were comparable to those of most protein/lipid emulsion edible films. The TS and %E values

were also comparable to those of low density polyethylene film and cellophane, respectively (Table 2.8).

2.4.5 Microstructure

SEM micrographs of whey protein/lipid emulsion films are shown in Figures 2.8, 2.9 and 2.10. Magnification of 400X was used for cross-sections of the films (Figure 2.8). The top surface (open to the environment while drying) of the films were magnified to 1,500X (Figures 2.9, 2.10), and these SEM micrographs were taken at a tilted angle (45°) to show a cross-sectional top surface of the films. SEM micrographs of the bottom surfaces, in contact with the casting surface while drying, of films were not shown here since no differences were observed among treatments.

In Figure 2.8, cross-sections of both WPI and WPC films are shown with no lipids (Figures 2.8a, d), with 4% BF (Figures 2.8b, e), and with 4% CW (Figures 2.8c, f). In overall comparison, WPC films showed a coarse protein matrix while WPI films appeared to be more compact and less coarse. This cross-sectional analysis showed that WPI films were more continuous and homogeneous (Figures 2.8a-c) than WPC films (Figures 2.8d-f). Differences in composition between WPC compared to that of WPI probably account for the loosely packed open film matrix as apparent in the microstructure, thus the previously reported lower TS of these films (Tables 2.1, 2.7).

Figures 2.9 and 2.10 shows top surfaces, lipid-oriented sides, of WPI and WPC films, respectively. In Figures 2.9a and 2.10a, top surfaces of WPI and WPC films without lipids (BF and CW) appeared to be smooth. Addition of lipids to both WPI and WPC films induced irregular morphology on the top surfaces (Figures 2.9b-e, 2.10b-e).

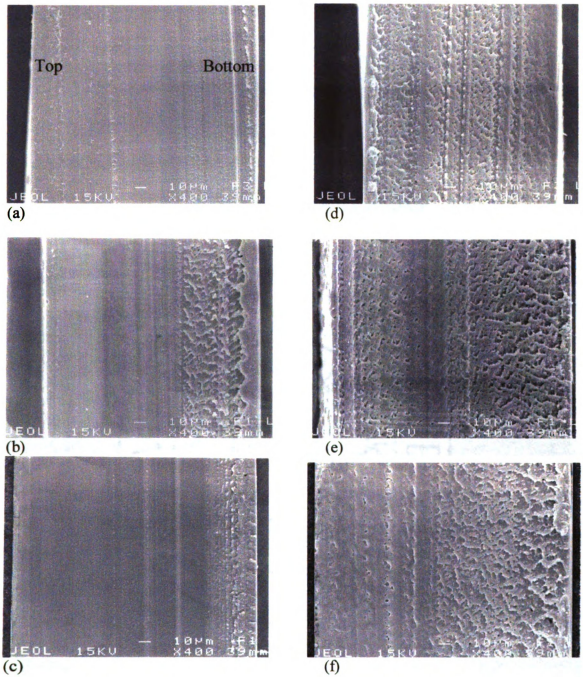
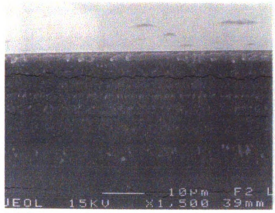


Figure 2.8. Scanning electron micrographs of cross-sections of whey protein/lipid emulsion films. Magnification 400X.

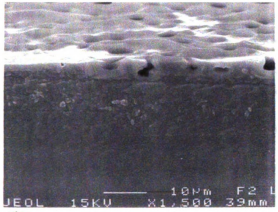
(a) IS; (b) IS-BF4%; (c) IS-CW4%; (d) CS; (e) CS-BF4%; (f) CS-CW4%.

I = whey protein isolate; C = whey protein concentrate; S = sorbitol;

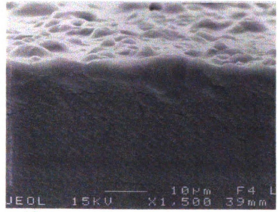
BF = butter fat; CW = candelilla wax



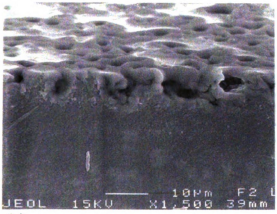
(a)



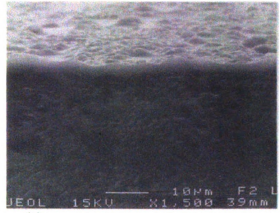
(b)



(d)



(c)

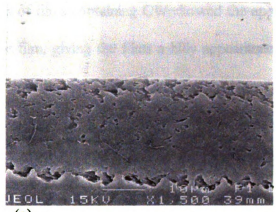


(e)

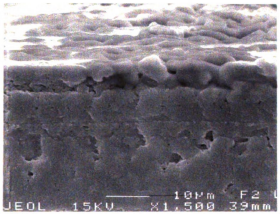
Figure 2.9. Scanning electron micrographs of top surfaces of whey protein isolate/lipid emulsion films. Magnification 1,500X.

(a) IS; (b) IS-BF2%; (c) IS-BF4%; (d) IS-CW4%; (e) IS-CW16%.

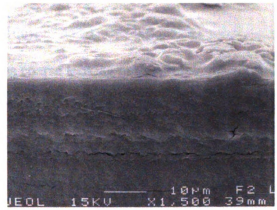
I = whey protein isolate; S = sorbitol; BF = butter fat; CW = candelilla wax



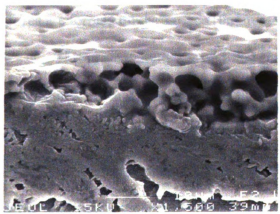
(a)



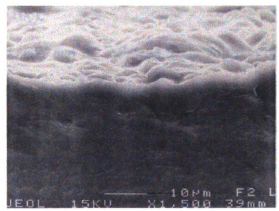
(b)



(d)



(c)



(e)

Figure 2.10. Scanning electron micrographs of top surfaces of whey protein concentrate/lipid emulsion films. Magnification 1,500X.

(a) CS; (b) CS-BF2%; (c) CS-BF4%; (d) CS-CW%4; (e) CS-CW16%.

C = whey protein concentrate; S = sorbitol; BF = butter fat; CW = candelilla wax

Top surface micrographs of films containing CW showed the appearance of wax droplets dispersed throughout the film, giving the films a hilly appearance, however, the structure appeared fairly compact (Figures 2.9d-e, 2.10d-e). No significant morphological differences could be seen among the top surface of films containing different levels of CW. On the other hand, increased BF contents can be observed by a BF layer distinctive from the top surfaces of the films (Figures 2.9b-c, 2.10b-c). As BF concentration increased in both WPI and WPC films, a thicker layer of BF and bigger void spaces were introduced on the top surfaces of the films (Figures 2.9c, 2.10c).

In particular, BF4%-added WPC film was coarse looking in the protein matrix and had open structures in lipid layer (Figures 2.8e, 2.10c). Earlier I reported this film to have the lowest TS (Table 2.7). This porous structure may also explain the high OP value obtained with these films compared to CW containing films (Figure 2.5). Apparently, oxygen molecules can more readily permeate through the film matrix when some holes and pores are formed on the surface (Figures 2.9c, 2.10c).

This present study showed that the lipids, CW and BF, oriented to the top side of dried films, which were exposed to the environment during drying. Greener and Fennema (1989a) found a similar morphology in methylcellulose/beeswax films. Beeswax was formed on the top surface of the films. Avena-Bustillos and Krochta (1993) observed the same tendency in the casein/lipid emulsion films. This phase separation probably occurred in whey protein/lipid emulsion films due to density differences between lipids and whey proteins, and responsible for the appearance of the dull and shiny sides of lipid added films (Figure 2.2). Moderate emulsifying ability of whey proteins may be responsible for this emulsion instability (McHugh and Krochta, 1994c; 1994e).

In summarizing the results of this chapter, addition of lipids, BF and CW, enhanced moisture barrier properties of the whey protein-based edible films. The most desirable WVP was observed with CW16%-added WPI films. BF and CW addition also improved oxygen barrier properties of the films. Whey protein type (WPI and WPC) did not affect barrier properties (WVP or OP) of the films. Protein, and lipid type and concentration influenced mechanical properties of the films. Overall, higher TS was observed with CW-added WPI films while BF-added WPC films showed higher %E. SEM micrographs illustrated relationship between the film's microstructure, and barrier and mechanical properties.

CHAPTER 3

FORCES INVOLVED IN THE FORMATION AND STABILITY OF WHEY PROTEIN/ LIPID EMULSION EDIBLE FILMS.

3.1 ABSTRACT

Whey protein isolate (WPI; 5%, w/v) or concentrate (WPC; 5%, w/v) films were plasticized with sorbitol (4.2-5%, w/v), and butter fat (BF; 0.2%, w/v) or candelilla wax (CW; 0.8%, w/v) was added into the film-forming solutions. Forces involved in formation and stability of the films were studied by determining their solubilities in 2-mercaptoethanol (2-ME; a disulfide bond-dissociating agent), urea (a hydrogen bond-dissociating agent and also a weak hydrophobic bond-dissociating agent) and sodium dodecyl sulfate (SDS; a hydrophobic bond-dissociating agent). Free sulfhydryl groups and disulfide bonds concentrations of the films were determined using Ellman's reagent 5,5'-dithiobis (2-nitrobenzoic acid) and disodium 2-nitro-5-thiosulfobenzoate, respectively. Additionally, hydrophobicity of the films was investigated using an extrinsic fluorescence probe 1-anilino-8-naphthalene sulfonic acid. Disulfide and hydrogen bonds, cooperatively, were the primary forces involved in the formation of whey protein/lipid emulsion films. Contribution of hydrophobic interactions was secondary. Sulfhydryl contents of the films ranged from 2.5 to 3.0, and 1.39 to 1.44 μmol/g of film for WPI and WPC films, respectively. Disulfide contents were not significantly different among treatments and ranged from 1.8 to 3.6 μmol/g of film. Hydrophobicity of the films increased with

incorporation of lipids. Although CW containing films were more hydrophobic than BF containing films, these differences were not statistically significant.

3.2 INTRODUCTION

Proteins such as wheat gluten, soy, corn zein, and whey proteins have been studied as film-forming materials in the production of protein-based edible films (Gennadios et al., 1994). For effective formation of films, extensive protein-protein interactions are necessary to form a continuous film network with sufficient mechanical strength. Protein-based film formation is thought to occur through protein polymerization and solvent evaporation at the surface of the aqueous dispersion (Gennadios and Weller, 1991). For instance, wheat proteins are capable of forming wheat gluten colloidal complex upon hydration in alkaline solutions. Intramolecular and intermolecular disulfide bonds in the gluten complex are cleaved and reduced to free sulfhydryl groups. Reformation of disulfide bonds occurs upon casting and drying to produce the film structure. Hydrogen bonds and hydrophobic interactions are also thought to contribute to film matrix (Gennadios et al., 1994). Similar to wheat protein-based films, soy protein-based films result due to the polymerization of heat denatured proteins through disulfide bonds. Hydrogen bonds and hydrophobic interactions are also important in maintaining the soy protein-based film's stability (Farnum et al., 1976). Zein, on the other hand, produces film structure primarily through hydrogen bonding and hydrophobic interactions. The role of disulfide bonds in the formation of the zein-based films is minimal due to its low cysteine content (Reiners et al., 1973).

Two primary proteins that make up whey proteins are β-lactoglobulin and α-lactoalbumin. Secondary and tertiary structures of these proteins are facilitated by hydrogen bonds. Intramolecular disulfide bonds also contribute to their stability (Brunner, 1981; Shimada and Cheftel, 1989). As the temperature of the solution is increased as in the case of film-forming process, polymerization of whey protein molecules occurs after the conformational change of their tertiary structures. The heat denaturation of whey proteins induces exposure of internal free sulfhydryl groups, promoting intermolecular disulfide bond formation. Free sulfhydryl groups also can be involved in interchange reactions with existing disulfide bonds upon heating and regenerate free sulfhydryl groups. These regenerated free sulfhydryl then react again in free sulfhydryl/disulfide bond interchange reactions. The heat treatment also results in exposed hydrophobic groups at the molecular surface (Shimada and Cheftel, 1989; Damodaran, 1996). This process may result in the formation of a protein network, which possesses sufficient mechanical strength to produce freestanding films upon drying (Cheftel et al., 1985; Gennadios et al., 1994).

Mahmoud and Savello (1993) studied forces involved in formation and stability of transglutaminase cross-linked whey protein-based films. Transglutaminase catalyzes the covalent binding of lysine with a glutamic acid residue, and generates intramolecular and intermolecular ε-(γ-glutaminyl)lysine cross-links. The transglutaminase cross-linked films produced by Mahmoud and Savello (1993) were insoluble in 2-mercaptoethanol (a disulfide bond-dissociating agent) and sodium dodecyl sulfate (a hydrophobic bond-dissociating agent), but were soluble in urea and guanidine hydrochloride (a hydrogen bond-dissociating agent and also a weak hydrophobic bond-dissociating agent). This led

them to believe that in addition to the covalent bonds formed by cross-linking, hydrogen bonding also contributed to the stability of these films. Limited information is available on forces influencing the structure of heat catalyzed whey protein-based films. Thus, the intent of this research was to gain an understanding of the molecular forces involved in the formation and stability of heat catalyzed whey protein-based and lipid emulsion films so that their properties can be improved or altered by enhancing or altering these interactions.

3.3 MATERIALS AND METHODS

3.3.1 Materials

Ellman's reagent [5,5'-dithiobis (2-nitrobenzoic acid) (DTNB)], 1-anilino-8-naphthalene sulfonic acid (ANS), 2-mercaptoethanol (2-ME), ethylenediaminetetraacetic acid (EDTA), and Tris-HCl were purchased from Sigma Chemical Co. (St. Louis, MO). Urea, trichloroacetic acid (TCA), and sodium sulfite were purchased from J.T. Baker Co. (Phillipsburg, NJ). Sodium dodecyl sulfate (SDS) was obtained from Pierce Co. (Rockford, IL).

3.3.2 Film preparation

Composition of the films prepared to study the effect of bond-dissociating agents, free sulfhydryl groups and disulfide contents, and hydrophobicity are shown in Table 3.1. The film preparation was carried out as described in section 2.3.2 of chapter 2 (Figure 2.1).

Table 3.1. Composition of whey protein/lipid emulsion edible films produced.

Treatments [1]	% w/v of aqueous solution			% w/w of protein	
	Protein [2]	Sorbitol	Lipid	Sorbitol	Lipid
IS	5	5.0	0	100	0
IS-BF4%	5	4.8	BF = 0.2	96	BF = 4
IS-CW16%	5	4.2	CW = 0.8	84	CW = 16
CS	5	5.0	0	100	0
CS-BF4%	5	4.8	BF = 0.2	96	BF = 4
CS-CW16%	5	4.2	CW = 0.8	84	CW = 16

[1] I = whey protein isolate; S = sorbitol; BF = butter fat; CW = candelilla wax; C = whey protein concentrate.

[2] whey protein isolate or whey protein concentrate

3.3.3 Solubility in bond-dissociating agents

The effect of bond-dissociating agents on each films solubility and stability was determined according to the method of Mahmoud and Savello (1993) with slight modifications. 2-ME has a free sulfhydryl group which can dissociate disulfide bonds (Stenesh, 1989). Urea breaks hydrogen bonds of proteins and interacts with unfolded proteins through hydrogen bonding altering the physical properties of water. Urea also can weaken hydrophobic bonds by altering the structures of water (Creighton, 1993; Lefebvre-cases et al., 1998). SDS is a hydrophobic bond-dissociating agent. SDS, an ionic detergent, has both hydrophobic and hydrophilic portions in its molecular structure, and can break hydrophobic interaction (Lapanje, 1978; Lefebvre-cases et al., 1998).

Film (30mg) was placed in 6ml of protein bond-dissociating agents: 0.1M 2-ME, 0.5M SDS and 6.6M urea. The samples were incubated at room temperature for 24h, then centrifuged at 9,000 x g for 20min (Biofuge 22R, Heraeus Instruments, Hanau, Germany). The total absorbance of the supernatant (2ml) was measured at 280nm (Spectronic 1001+, Milton Roy Co., Rochester, NY). Proteins exhibit strong absorption at UV 280 nm due to tryptophan and tyrosine residues (Chang, 1994). The supernatant (2ml) was then mixed with 6% TCA (2ml), allowed to react for 30 min and centrifuged at 9,000 x g for 20min. The A_{280} of the supernatant represented non-protein absorbance. Soluble protein absorbance was calculated as following:

Soluble protein absorbance = total absorbance - non protein absorbance (17)

3.3.4 Determination of free sulfhydryl group and disulfide bond contents

Free sulfhydryl groups and disulfide bonds in whey protein/lipid emulsion films were determined according to Rangavajhyala et al. (1997) with slight modifications. This procedure was developed based upon methods of Thannhauser et al. (1987) and Chan and Wasserman (1993). Ellman's reagent 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) and disodium 2-nitro-5-thiosulfobenzoate ($NTSB^{2-}$) were used for the determination of free sulfhydryl group and disulfide bond contents, respectively. Reaction with these color reagents yield soluble 2-nitro-5-thiobenzoate anion (NTB^{2-}).

Since $NTSB^{2-}$ was not commercially available, DTNB was used to synthesize $NTSB^{2-}$ according to Thannhauser et al. (1987). DTNB (0.2g) was dissolved in 1M sodium sulfite (20ml) at 38°C, and pH was adjusted to 7.5. While keeping this solution at 38°C, oxygen was bubbled into the solution until the bright red solution turned to a pale yellow (about 45 min). This stock solution was then used to prepare NTSB assay solution.

To assay free sulfhydryl group content in the films, a 10 mg of film was added into 1 ml of reaction buffer containing 8 M urea, 10 mM DTNB, 3 mM EDTA, 1% SDS, and 0.2 M Tris-HCl, pH 8.0. The reaction mixture was held at room temperature (~ 23°C) for 15 min. To remove particulate matter, the mixture was centrifuged at 13,600 x g for 10min. A 0.2ml aliquot of supernatant was removed and diluted with 1.8 ml of 8M urea, 3mM EDTA, 1% SDS, and 0.2M Tris-HCl, pH 8.0. Then its absorbance was measured at 412 nm (Spectronic 1001+, Milton Roy Co.). Sulfhydryl content was calculated by using the extinction coefficient of NTB (13,600 $M^{-1}cm^{-1}$) at 412nm.

To assay disulfide bond content in the films, a 10 mg of film was added into 1 ml of reaction buffer containing 8 M urea, 10 mM $NTSB^{2-}$, 0.1 M sodium sulfite, 3 mM EDTA, 1 % SDS, and 0.2 M Tris-HCl, pH 9.5. The reaction mixture was held in the dark chamber for 25 min at room temperature (~ 23°C), then centrifuged at 13,600 x g. A 0.2ml aliquot of supernatant was removed and diluted with 1.8ml of 8M urea, 0.1 M sodium sulfite, 3mM EDTA, 1% SDS, and 0.2 M Tris-HCl, pH 9.5. Its absorbance was measured at 412nm, and referred to as total sulfhydryl group content. Disulfide bond content was determined as following:

Disulfide bond =

$$1/2 \times (|\text{total sulfhydryl group content} - \text{free sulfhydryl group content}|) \quad (18)$$

3.3.5 Determination of hydrophobicity

Hydrophobicity of the films was determined using an extrinsic fluorescence probe 1-anilino-8-naphthalene sulfonic acid (ANS) according to Lakkis and Villota (1992). Standard plot of hydrophobicity was established by measuring hydrophobicity of ten different concentrations (0.05mg/ml to 0.5mg/ml of films in phosphate buffer, pH 7). To obtain film-dispersed solutions, 500mg of film pieces were dissolved into 10ml of ultra purified H_2O with sodium azide (0.02%, to prevent microbial growth) for 24 hr at room temperature (~ 23°C).

To assay intensity of the film hydrophobicity, different concentrations of film dispersions and ANS (10µl, 8mM in 0.1 M phosphate buffer) were added to a cuvette. Phosphate buffer, pH 7.0, was added to bring the total volume to 2ml. Relative fluorescence intensity (RFI) of samples was measured using a Model 4800

spectrofluorometer (SLM Instruments, Urbana-Champaign, IL) connected to a data acquisition and operating system (On-Line Instrument Systems, Bogart, GA). Fluorescence measurement was done with semi-micro quartz fluorescence cuvettes (4 x 10 mm) held in a thermostable block (22°C). The excitation wavelength was 390nm (excitation wavelength of ANS) and emission was scanned (400-600nm) with slit width of 4nm. The fluorescence intensity based on emission maximum wavelength at 486nm was measured. RFI of films in phosphate buffer (pH 7.0) was also measured without ANS. The net RFI for each sample was obtained by subtracting the RFI without ANS.

The initial slope (S_o) of the RFI versus film concentrations plot was determined and referred to as a hydrophobicity index of the sample. Under conditions with excessive probe, the S_0 value has shown a close correlation to the hydrophobicity of proteins (Kato and Nakai, 1980; Yildirim et al., 1996; Haskard and Li-Chan, 1998).

3.3.6 Statistical Analysis

All experiments were replicated three times in a randomized block experiment. A new film forming solution and new set of films were prepared for each replicate. Statistical analysis were made using Sigma Stat 2.0 (Jandel Corp., San Rafael, CA) and the appropriate comparisons were done using the Student-Newman-Keuls method for multiple comparisons.

3.4 RESULTS & DISCUSSION

3.4.1 Solubility of the films in bond-dissociating agents

Solubilities of whey protein/lipid emulsion films in bond-dissociating agents, 2-ME, urea, and SDS, are shown in Figure 3.1. Relatively higher protein solubilities were observed when 2-ME (a disulfide bond-dissociating agent) and urea (a hydrogen bond-dissociating agent and also a weak hydrophobic bond-dissociating agent) were the solvents rather than SDS (a hydrophobic bond dissociating-agent).

The strong effect of 2-ME on protein dissociation in WPI and WPC films with and without lipids reflected the important contribution of disulfide bonds to the formation and stability of these films. Disulfide bond formation is promoted during the heat treatment of the film-forming solutions and then during drying of the films. These results are consistent with those of Pérez-Gago et al. (1999). They prepared WPI-based films with or without heat treatment of the film-forming solutions, and compared the mechanical properties, tensile strength (TS) and elongation at break (%E), of the films. Reportedly, TS and %E of the WPI-based films were much lower ($p < 0.05$) when the films were prepared without heat treatment compared to those of the heat denatured WPI-based films. They concluded that the disulfide bond formation through heat-denaturing process was important for the formation of the WPI-based film network and the film's structural integrity.

A similar action of urea as with 2-ME on protein dissociation in both whey protein type films suggested the presence of hydrogen bonds in the stabilization of the film network (Figure 3.1). Urea is believed to alter the physical properties of water; at high concentrations, it hydrogen bonds to water in an aqueous system, thus, disrupting the

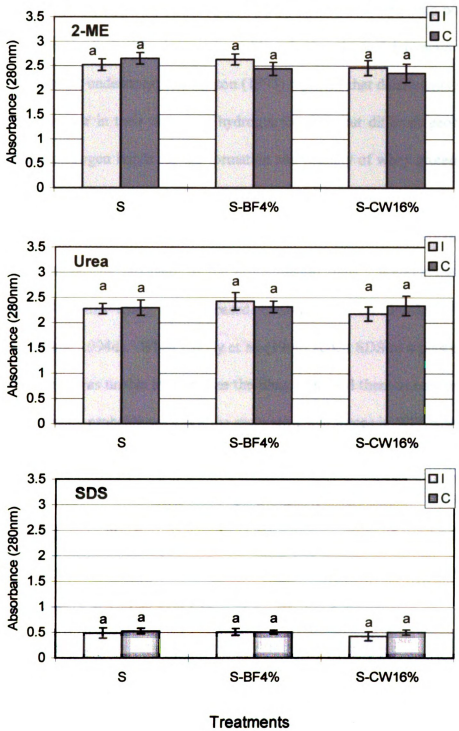


Figure 3.1. Solubility of whey protein/lipid emulsion edible films in protein bond-dissociating agents. I = whey protein isolate; C = whey protein concentrate; S = sorbitol; BF = butter fat; CW = candelilla wax.

usual hydrogen bond network of the aqueous system. Urea also interacts with polar and nonpolar surfaces more favorably than water, although the physical interactions with nonpolar groups are not understood. Creighton (1993) reported that denaturants such as urea are similar to water in their degree of hydrogen bonding but different geometries. The importance of hydrogen bonds for the formation and stability of whey protein-based films was expected since plasticizer (sorbitol) must be added to produce flexible films. Sorbitol reduces protein-protein interactions by forming hydrogen bonds with polypeptides, thus increasing flexibility of the protein-based films. Without the aid of plasticizer (sorbitol in this case), protein-based films become too brittle to handle (McHugh and Krochta, 1994d). When Fairley et al. (1996) added SDS as a plasticizer to WPI-based films, SDS was unable to plasticize the films. This led them to conclude that hydrophobic interactions probably were not the main attractive forces in WPI-based films since a hydrophobic bond-dissociating agent SDS was not an effective plasticizer for the films. The lower protein solubility with SDS (Figure 3.1) may indicate the contribution of hydrophobic interactions to film formation and stability were not as significant as disulfide or hydrogen bonds.

Solubilities of whey protein-based films in protein bond-dissociating agents were only determined on transglutaminase cross-linked films by Mahmoud and Savello (1993) and Yildirim and Hettiarachchy (1998). Their studies showed that whey protein-based films were stable in 2-ME while the films were highly soluble in urea. They concluded that the result suggested the insignificance of disulfide bonds and the significance of hydrogen bonds for the formation and stability of the films. However, contribution of hydrophobic bonds for the formation and stability of the films were different. Mahmoud and Savello

(1993) reported that SDS was unable to solubilize transglutaminase cross-linked whey protein-based films and concluded that hydrophobic bonds' contribution was minimal. On the other hand, solubility of transglutaminase cross-linked whey protein-based films in SDS was higher than with urea in the study by Yildirim and Hettiarachchy (1998), which suggested the importance of hydrophobic bonds for the formation and stability of the films. Unfortunately, no studies investigated solubility of heat-catalyzed whey protein-based films in protein bond-dissociating agents other than this present study.

In this present study, the film's solubilities in bond-dissociating agents, 2-ME, urea and SDS, showed no relevancy to their mechanical properties (TS and %E; Figure 3.1, Table 2.7). Although the TS and %E values of the films were significantly ($p < 0.05$) changed upon addition of BF (4%, w/w of protein) and CW (16%, w/w of protein), no differences were observed in the films' solubilities in the different bond-dissociating agents. Protein type (WPI and WPC) also did not affect the films' solubilities in bond-dissociating agents. A protein content difference (~10%) between WPI and WPC, 90 and 80%, respectively, perhaps was not significant enough to affect chemical bond formation in the films. Neither there was a relationship between chemical bonds in the films, and their moisture and oxygen barrier properties. In Figure 3.1, solubilities of the films in bond-dissociating agents (2-ME, urea and SDS) were not different among treatments while water vapor and oxygen permeabilities of the films were significantly changed ($p < 0.05$) depending on the protein and lipid type and concentration (Table 2.3, 2.5). Structural and chemical natures of polymers are thought to play significant roles in the permeability of the films (McHugh and Krochta; 1994a). Results from this study showed that chemical bond formation among treatments was not statistically different (Figure 3.1).

Perhaps barrier properties of whey protein/lipid emulsion films are more influenced by structural differences, and surface morphology or matrix density of the films.

3.4.2 Free sulfhydryl group and disulfide bond contents of the films

The free sulfhydryl group and disulfide bond contents of whey protein/lipid emulsion films are shown in Tables 3.2. Free sulfhydryl group content of WPI film was higher ($p < 0.05$) than those of WPC films, 2.50-3.24 and 1.39-1.44 μmol/g of film, respectively. This is probably due to the differences in the total protein amounts between WPI and WPC, 90 and 80%, respectively (Table 2.1). Lipid type (BF or CW) had no effect on free sulfhydryl group contents of the WPI and WPC films. This was expected since protein amounts were kept the same (50% w/w, dry basis) throughout the treatments (Table 3.1).

Disulfide contents of the WPI and WPC films ranged from 2.75 to 3.58, and 1.84 to 2.35μmol/g of film, respectively (Table 3.2). However, no statistical differences were observed among treatments. Lipid type (BF and CW) also did not affect disulfide contents of the films. Although there were no statistical differences in disulfide contents of the films, a relationship was observed between disulfide contents of the films and TS of the films. Lower disulfide contents of WPC films (1.84-2.35μmol/g of film) coincided with lower TS values (2.8-5.1 MPa) of the films compared to those of WPI films, while higher disulfide contents of WPI films (2.75-3.58μmol/g of film) agreed with higher TS values (3.7-7.9 MPa) of the films (Tables 2.7, 3.2). Similar results were reported by Were et al. (1999) when they investigated disulfide contents of soy-wheat gluten composite films. Reportedly, the highest TS was observed with the film that exhibited the highest disulfide

Table 3.2. Free sulfhydryl group and disulfide bond contents of whey protein/lipid emulsion edible films.

Treatments [1]	SH (μmol/g of film)	S-S (μmol/g of film)
IS	3.04 ± 0.17 [a]	2.75 ± 0.41 [a]
IS-BF4%	2.50 ± 0.39 [a]	3.01 ± 0.45 [a]
IS-CW16%	2.89 ± 0.31 [a]	3.58 ± 0.48 [a]
CS	1.39 ± 0.37 [b]	2.33 ± 0.69 [a]
CS-BF4%	1.44 ± 0.26 [b]	1.84 ± 1.02 [a]
CS-CW16%	1.39 ± 0.31 [b]	2.35 ± 0.90 [a]

[a-b] Different letters columnwise denote significant difference ($p < 0.05$), n=3 for all treatments.
[1] I = whey protein isolate; S = sorbitol; BF = butter fat; CW = candelilla wax; C = whey protein concentrate

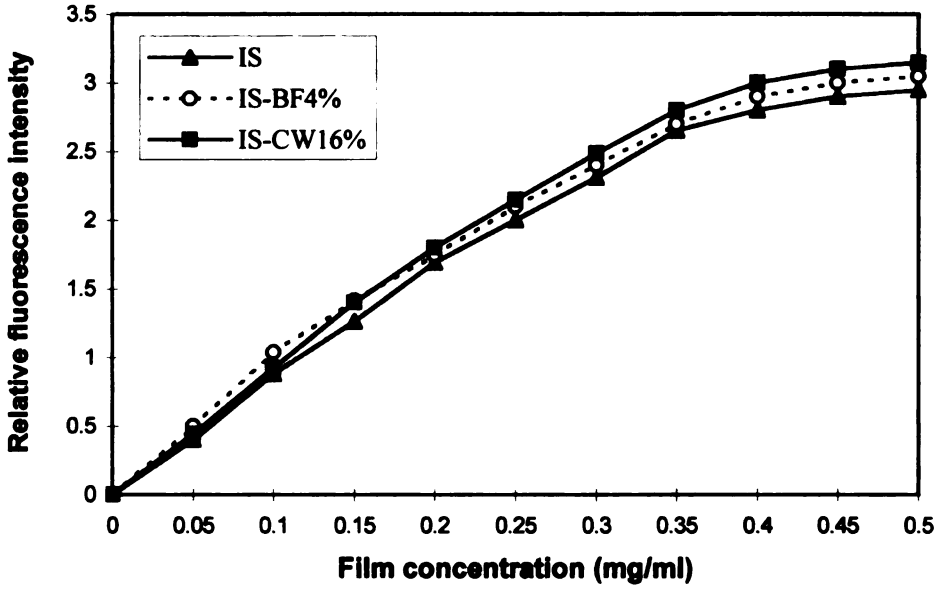
content. They also suggested that additional introduction of disulfide bonds to films may increase TS of the films, and suggested use of a free sulfhydryl group containing compound like cysteine in film-forming solutions to induce free sulfhydryl/disulfide interchange reaction and rearrangement of disulfide bonds.

3.4.3 Hydrophobicity of the films

Relative fluorescence intensities (RFI) of ANS at 486nm with varying concentrations of whey protein/lipid emulsion films, 0.05 to 0.5 mg/ml in phosphate buffer, are shown in Figure 3.2. These RFI versus film concentrations plots were used to obtain initial slope (S_o), hydrophobicity values, of the films. The S_o values of whey protein/lipid emulsion films ranged from 8.40 to 9.38 (Table 3.3). Addition of lipids, BF and CW, resulted in increased hydrophobicity of WPI films from 8.40 to 8.61 and 9.05, respectively. A similar trend was observed for the hydrophobicity of WPC films. Higher S_o values for BF or CW added WPC films were obtained compared to that of the film without lipids, 8.44, 8.52 and 9.38, respectively. However, these differences were not statistically significant.

Although no statistical differences were observed among treatments, overall, CW16%-added WPI and WPC films exhibited higher hydrophobicity values compared to those of the films without lipids and with BF4% (Table 3.3). This was expected since CW belongs to the non-polar lipid group (Callegarin et al., 1997). Incorporation of BF4% to WPI and WPC films was not as effective as CW to increase hydrophobicity of the films. This is probably due to the nature of BF. About 98% of BF is composed of triacylglycerols, which make BF a polar lipid (Sax and Lewis, 1987).

(a)



(b)

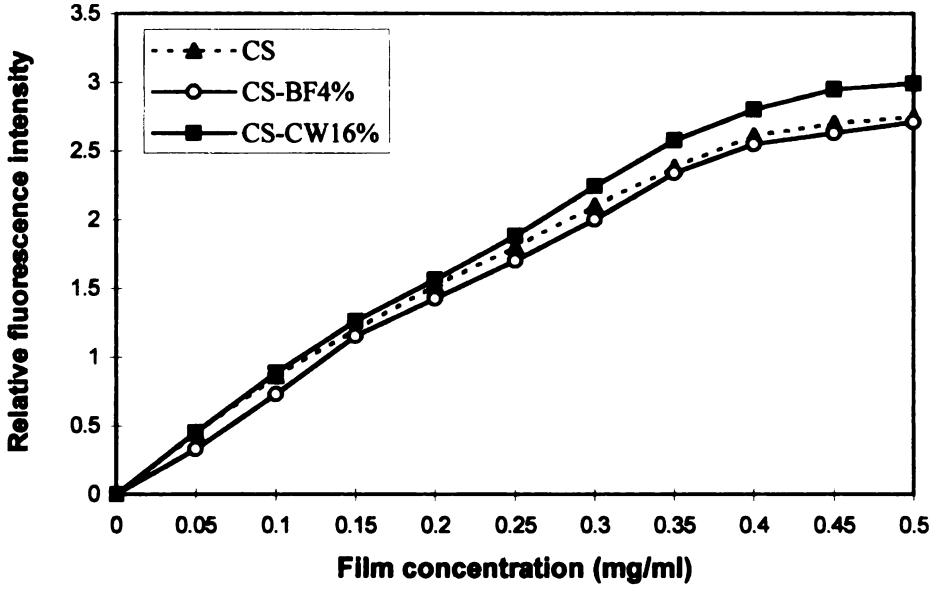


Figure 3.2. Relative fluorescence intensity (RFI) of ANS at 486nm with concentration of whey protein/lipid emulsion edible films in the range of 0.05 to 0.5 mg/ml in aqueous phosphate buffer (Data shown here are the average of three replicates).
(a) whey protein isolate films; (b) whey protein concentrate films;
I = whey protein isolate; C = whey protein concentrate;
S = sorbitol; BF = butter fat; CW = candelilla wax.

Table 3.3. Hydrophobicity (S_o) values of whey protein/lipid emulsion edible films.

Treatments [1]	S_o
IS	8.40 ± 0.47 [a]
IS-BF4%	8.61 ± 0.35 [a]
IS-CW16%	9.05 ± 0.72 [a]
CS	8.44 ± 0.32 [a]
CS-BF4%	8.52 ± 0.46 [a]
CS-CW16%	9.38 ± 0.60 [a]

[a-b] Different letters columnwise denote significant difference ($p < 0.05$), n=3 for all treatments.
[1] I = whey protein isolate; S = sorbitol; BF = butter fat; CW = candelilla wax; C = whey protein concentrate.

This trend in hydrophobicity of the films coincided with reduced ($p < 0.05$) water vapor permeability (WVP) of the films upon addition of lipids (Tables 2.3, 3.3). For instance, the highest WVP of WPI films was observed with WPI films without lipids added which exhibited the lowest hydrophobicity value, 28.5 g.mm/m^2.day.kPa at 80% RH and 8.40, respectively. On the other hand, CW16%-added WPI films showed the lowest WVP 20.7 g.mm/m^2.day.kPa had the highest hydrophobicity value 9.05. A similar trend was observed with WPC films' WVP and hydrophobicity values (Tables 2.3, 3.3). Unfortunately, no other studies are found reporting hydrophobicity of protein/lipid emulsion films.

In summary, the results of this study demonstrated that disulfide and hydrogen bonds were the main forces involved in the formation and stabilization of whey protein/lipid emulsion edible films. The contribution of hydrophobic interactions to film formation and stability were secondary. Protein type (WPI and WPC) affected the free sulfhydryl group contents of the films: 2.50-3.04 μmol/g of film for WPI films, and 1.39-1.44 μmol/g of film for WPC films. Although as a general trend WPI films had higher disulfide contents than WPC films, no statistical differences were observed among treatments. Hydrophobicity of the films increased with addition of lipids. CW-added films were more hydrophobic than films containing BF, however, these differences were not statistically significant.

CHAPTER 4

THERMAL PROPERTIES, HEAT SEALABILITY AND SEALING PROPERTIES OF WHEY PROTEIN/ LIPID EMULSION EDIBLE FILMS.

4.1 ABSTRACT

Whey protein isolate (WPI; 5%, w/v) films were plasticized with sorbitol (4.2-5%, w/v) and glycerol (2.7-3.5%, w/v), and butter fat (0.2%, w/v) or candelilla wax (0.8%, w/v) was added to produce whey protein/lipid emulsion edible films. Thermal properties of the film-forming components and of the films were studied using Differential Scanning Calorimetry (DSC). DSC analysis results were used to optimize heat-sealing conditions of the films. The effect of heat-sealing temperature, pressure, and dwell time on seal strength were investigated. Electron spectroscopy for chemical analysis (ESCA) was used to study the nature of interfacial interactions in heat sealed whey protein/lipid emulsion edible films. All films were heat sealable. The seal strengths of the films ranged from 110 to 323 N/m. Pressure and dwell time variation did not affect the seal strength of the films. However, the plasticizer type influenced heat-sealing temperature of the films, 130°C for sorbitol-plasticized films and 110°C for glycerol-plasticized films. ESCA spectra showed main components on the surfaces of unsealed and heat sealed films and gave evidence that hydrogen and covalent bonds involving C-O-H and N-C components, respectively, may be the main forces responsible for the sealed joint of whey protein-based films. Model structures for the interfacial interactions of the films upon heat-sealing were proposed.

4.2 INTRODUCTION

Edible packaging materials have received much interest in recent years since they provide unique and new opportunities for food processing and product development. In chapter 2, development, barrier and mechanical properties of whey protein/lipid emulsion edible films were reported. Some proposed applications of these protein-based edible films include pouches or sachets to package individual portions of dry ingredients, such as beverage mixes or soups for convenience. Other possible applications include 'ingredient delivery systems' that deliver pre-measured ingredients during processing operations, thus, offering convenience and preventing human error in weighing and handling (Debeaufort et al., 1998).

Sealability and sealing properties of materials are important in development of pouches sachets or 'ingredient delivery systems'. Application of heat is widely used in the packaging industry to seal polymer films. Seal properties of a film are typically influenced by sealing temperature, pressure, and dwell time. However, sealing temperature of the films has been reported to be most important in influencing seal strength (Meka and Stehling, 1994). Upon application of heat, surface regions of crystalline polymer melt and application of pressure leads to diffusion and entanglements of the melted surface. The intermolecular diffusion across the joint surfaces is a necessary step to give sufficient seal strength to the sealed films. The diffusion step requires time. After cooling, recrystallization occurs producing a heat-sealed joint (Yeh and Benatar, 1997; Mueller et al., 1998). This joint formation occurs through interfacial interactions on the polymer surface, which is dependent on the surface chemistry of the polymer (Allen, 1987).

Few studies have demonstrated heat sealability of edible films. Ninomiya et al. (1990) reported on heat sealability and seal strength of carageenan-based edible films. Glycerol- and sorbitol-plasticized carageenan-based films were heat sealable, and had seal strength of 137 and 130 N/m, respectively. Chick (1998) investigated heat sealability of lactic acid casein-based films. The films were heat sealable at around 107-120°C. Seal strength of the films ranged from 153 - 247 N/m. No studies have been reported on the mechanism involved in heat-sealing of protein-based edible films.

Electron spectroscopy for chemical analysis (ESCA) is a useful tool that provides qualitative and quantitative characterization of the near surface regions of materials (Cayless, 1991). Wu et al. (1995) investigated effects of ammonia plasma treatment on LDPE and HDPE surfaces to enhance seal strength of the two polymers. Surface analyses by ESCA revealed interactions occurred between nitrogen and oxygen containing functional groups on the ammonia plasma treated polymer surfaces. Possart and Dieckhoff (1999) also employed ESCA to study the surface of polycyanurates to determine which chemical structures are capable of interfacial interactions with various substrate surfaces. Reportedly, hydrogen bonds involving OH-groups were responsible for the interactions at the interfacial region.

Much of the research on protein-based edible films until now has focused on their development, determining their barrier and mechanical properties. Very little information is available on their thermal properties. There is no information available on heat sealability and seal properties of whey protein-based edible films. In this study, whey protein isolate films were plasticized with sorbitol and glycerol, and butter fat and candelilla wax were incorporated to produce whey protein/lipid emulsion films. The intent

was to determine thermal properties of the films using differential scanning calorimetry to optimize heat-sealing temperatures. Heat sealability and seal strength of the films were determined at various temperatures, pressures and dwell times to obtain optimum sealing conditions. Surface chemical properties of the unsealed and sealed film were investigated using ESCA to gain an understanding for the interfacial interactions in the formation of the sealed joint with whey protein/lipid emulsion films. In addition, model structures for interfacial interactions of the films were proposed to illustrate bonding formation on the surfaces of the films upon heat-sealing.

4.3 MATERIALS AND METHODS

4.3.1 Materials

Whey protein isolate (ALACEN 895) was obtained from New Zealand Milk Products (North America) Inc., (Santa Rosa, CA). Table 4.1 shows the composition of the whey proteins used in the production of edible films in this study. Its protein content, 93.2%, was confirmed using AOAC 930.29 Kjeldahl method (AOAC, 1990). Glycerol was purchased from J.T. Baker Co. (Phillipsburg, NJ). D-Sorbitol, candelilla wax, butter fat and NaOH were purchased from various sources as mentioned in chapter 2 section 2.3.2.

4.3.2 Film preparation

Edible film forming solutions were prepared by mixing whey protein isolate (WPI) (5%, w/v) with sorbitol or glycerol, 2N NaOH (to adjust pH), and distilled H_2O to a final

Table 4.1. Composition of whey proteins used[1].

Components	Whey protein isolate ALACEN 895
Protein (N x 6.38) %	93.5
Ash %	1.6
Moisture %	4.0
Fat %	<1.0
Lactose %	<1.0
pH[2]	6.8

[1] Values based on specification provided by New Zealand Milk Products (N. America) Inc.
[2] 5% at 20°C

volume of 100ml. Rests of the procedures were the same as described in section 2.3.3 of chapter 2 (Figure 2.1). Table 4.2 shows the composition of the films. Thicknesses of the films were measured using a TMI model 549 M micrometer (Testing Machines, Inc., Amityville, NY). Thicknesses of sorbitol- and glycerol-plasticized WPI films were 140 ± 19 and 120 ± 15 μm, respectively.

4.3.3 Thermal analysis of the films

Differential scanning calorimetry (DSC) was employed to determine thermal properties and processing conditions of whey protein/lipid emulsion film. Thermal transition temperatures of the film forming components and films were evaluated. A Du Pont 2920 DSC unit (Wilmington, DE) was used to measure the differential temperature and enthalpy change (ΔH). Approximately 10mg of sample was weighed and sealed in an aluminum sample pan (TA Instruments, Newcastle, DE) by an encapsulating press. Samples were heated from 0°C to 250°C with increase in temperature of 20°C/min. An empty sample pan was used as a reference. The DSC cell was flushed with nitrogen at 20 ml/min to maintain an inert environment during the measurement. The transition temperatures of the film forming components and films were determined by the General V 4.1 C software program (Wilmington, DE), which controlled the DSC unit. Onset (T_o) and peak (T_p) temperatures were assigned according to ASTM D-3418 "*Standard test method for transition temperature of polymers by thermal analysis*" (ASTM, 1997).

Thermogravimetric Analysis (TGA) was used to determine weight loss of whey protein powder during heating under conditions close to that of the DSC experiment. TGA is a technique in which the mass of a substance is measured as a function of

Table 4.2. Composition of whey protein/lipid emulsion edible films produced.

Treatments [1]	% w/v of aqueous solution			% w/w of protein	
	Protein [2]	Plasticizer	Lipid	Plasticizer	Lipid
IS	5	5.0	0	100	0
IS-BF4%	5	4.8	BF = 0.2	96	BF = 4
IS-CW16%	5	4.2	CW = 0.8	84	CW = 16
IG	5	3.5	0	70	0
IG-BF4%	5	3.3	BF = 0.2	66	BF = 4
IG-CW16%	5	2.7	CW = 0.8	54	CW = 16

[1] I = whey protein isolate; S = sorbitol ; BF = butter fat ; CW = candelilla wax ; G = glycerol
[2] whey protein isolate

temperature or time (ASTM, 1997). A Du Pont 2200 TGA unit (Wilmington, DE) was employed and approximately 10mg of WPI powder was prepared as described in DSC procedure. A sample was heated from 20 to 150°C with increase in temperature of 20°C/ min under a nitrogen atmosphere. The mass of the sample was measured concurrently.

4.3.4 Determination of seal strength

Film samples were cut into strips of 7.62cm x 2.54cm using a Precision sample cutter (Thawing Albert Instrument Co., Philadelphia, PA). Two layers of film strips were sealed together using a thermal heat sealer Model-12ASL (Sencorp System Inc., Hyannis, MA). The seal area was 2.54cm x 1.5cm. Three different seal temperatures 110, 120 and 130°C were investigated. These temperatures were selected based on DSC results. Dwell times were 1 or 3 seconds, with a seal pressure of 40 or 60psi. All sealed specimens were conditioned (23 ± 2°C, 50 ± 5 % RH) for 48 hours prior to testing for strength of the seal.

Seal strength of the films were determined using a ASTM F-88 "*Standard test method for seal strength of flexible barrier materials*" (ASTM, 1997). Tests were conducted using the Instron Universal Testing Machine Model 2401 (Instron Corp., Canton, MA) at 23 ± 2°C and 50 ± 5% RH. Each leg of the specimen was clamped into the testing machine. The distance between clamps was 5.08 cm with a loading rate of 25.4 cm/min. During the test, each end of the sealed film was held perpendicularly to the direction of pull as the specimen was stressed. The maximum force required to cause seal failure were reported in newtons/meter (N/m). Seal strength was calculated from the following equation:

$$\text{Seal strength} = \text{load} / w \quad (19)$$

load = peak force

w = film width

4.3.5 Surface analysis of unsealed and sealed films using Electron Spectroscopy for Chemical Analysis (ESCA)

Whey protein/lipid emulsion films were sealed at 110°C for 1 sec at pressure of 40psi. The surface elemental compositions and bonding distributions of unsealed, and sealed films were determined using ESCA with a PHI 5400 ESCA lab workstation (Physical Electronics, Eden Prairie, MN). A 15 mm diameter of circular sealed or unsealed film was placed in sample holder and monochromatic X-rays were used as the radiation source. All spectra were collected using a Mg anode operated at a power of 300 W with an analyzer pass energy of 33 eV. An electron kinetic energy analyzer plotted the intensity of the emitted photoelectrons according to their binding energies. The optimum spot size for the conditions used in this experiments was 1 mm diameter aperture.

The shape of the spectra indicated that no compensation for differential surface charging was needed. The bonding scale was calibrated to 284.6 eV for the main C1s (C-H) feature. Spectra were run in both low resolution (survey scan) and high resolution modes for the C1s, O1s and N1s regions. Elemental compositions were calculated from the survey scan spectra. Chemical information indicating changes in the surface of polymers was elucidated by curve-fitting the carbon 1s (C1s), nitrogen 1s (N1s), and oxygen 1s (O1s) spectra. Curve-fitting defined and interpreted the carbon chemistry as detected at the sample surface by allowing the user to distinguish overlapping features

within the spectral envelope. The spectra were fit with a Lorentzian-Gaussian mix Voigt profile function using a nonlinear least-square curve-fitting program PHI PC Explorer Software multipack (Physical Electronics, Eden Prairie, MN). The resulting curve fits have levels of experimental error of approximately 5%.

4.3.6 Statistical analysis

The seal strength experiments were replicated three times in a randomized block experiment. A new film forming solution and new set of films were prepared for each replicate. Statistical analysis were made using Sigma Stat 2.0 (Jandel Corp., San Rafael, CA) and the appropriate comparisons were made using the Student-Newman-Keuls method for multiple comparisons. DSC and ESCA were conducted on one set of each replicate. Representative data were selected for presentation in this chapter.

4.4 RESULTS & DISCUSSION

4.4.1 Thermal properties of the films

DSC results of the film forming components, and sorbitol- and glycerol-plasticized WPI films are shown in Figures 4.1, 4.2 and 4.3, respectively. Onset (T_o) and peak (T_p) temperatures were assigned according to ASTM D-3418 (Tables 4.3, 4.4). WPI powder exhibited a broad endothermic peak of *first-order* transition between 125 and 173°C, similar to the distinctive melting transition characteristic of semicrystalline polymers, suggesting that WPI may be a partially amorphous semicrystalline polymer. It is thought that all polymers have at least some amorphous material (Rosen, 1982). WPI powder had

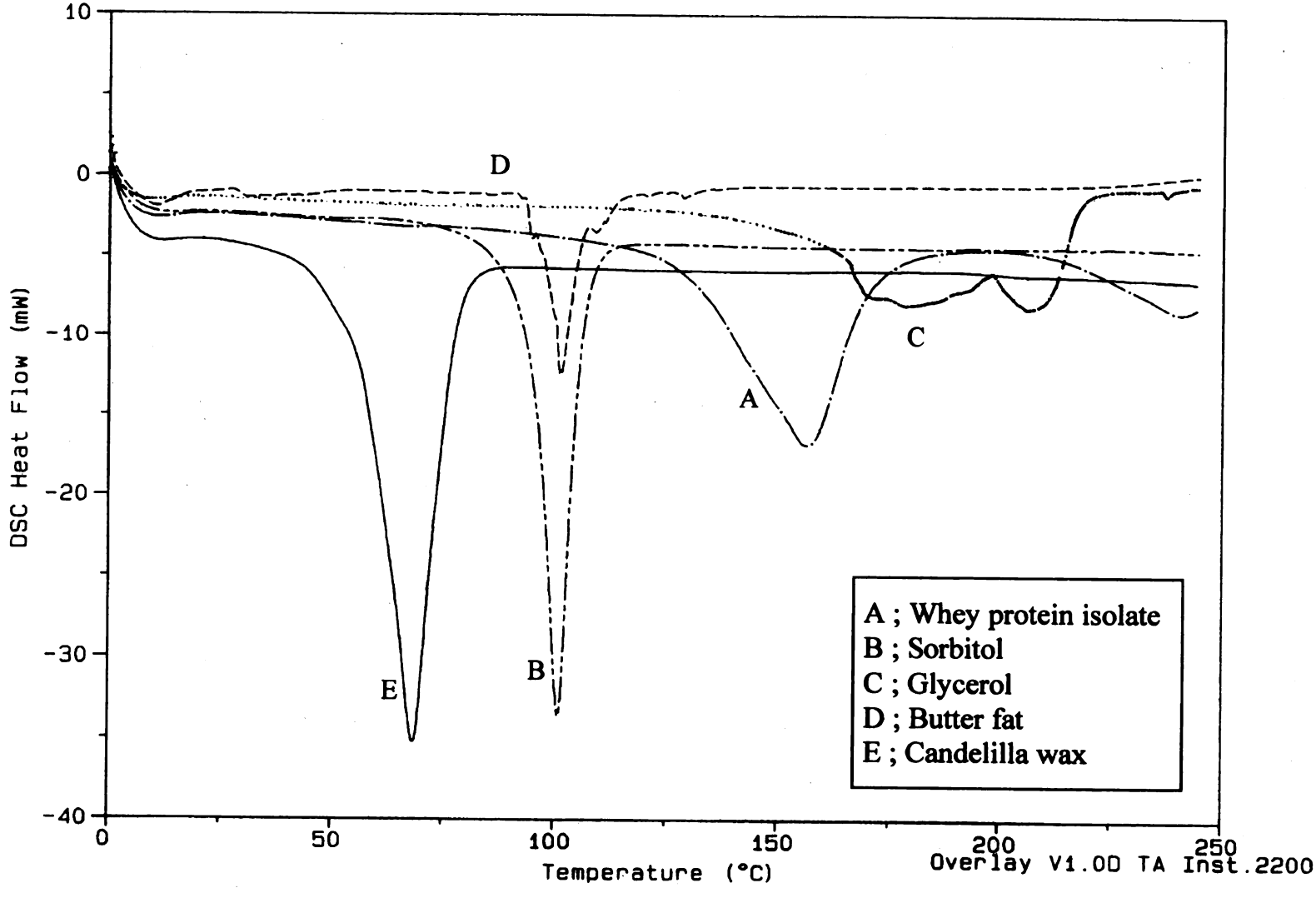


Figure 4.1. Differential Scanning Calorimetry thermogram of film components (20°C/min).

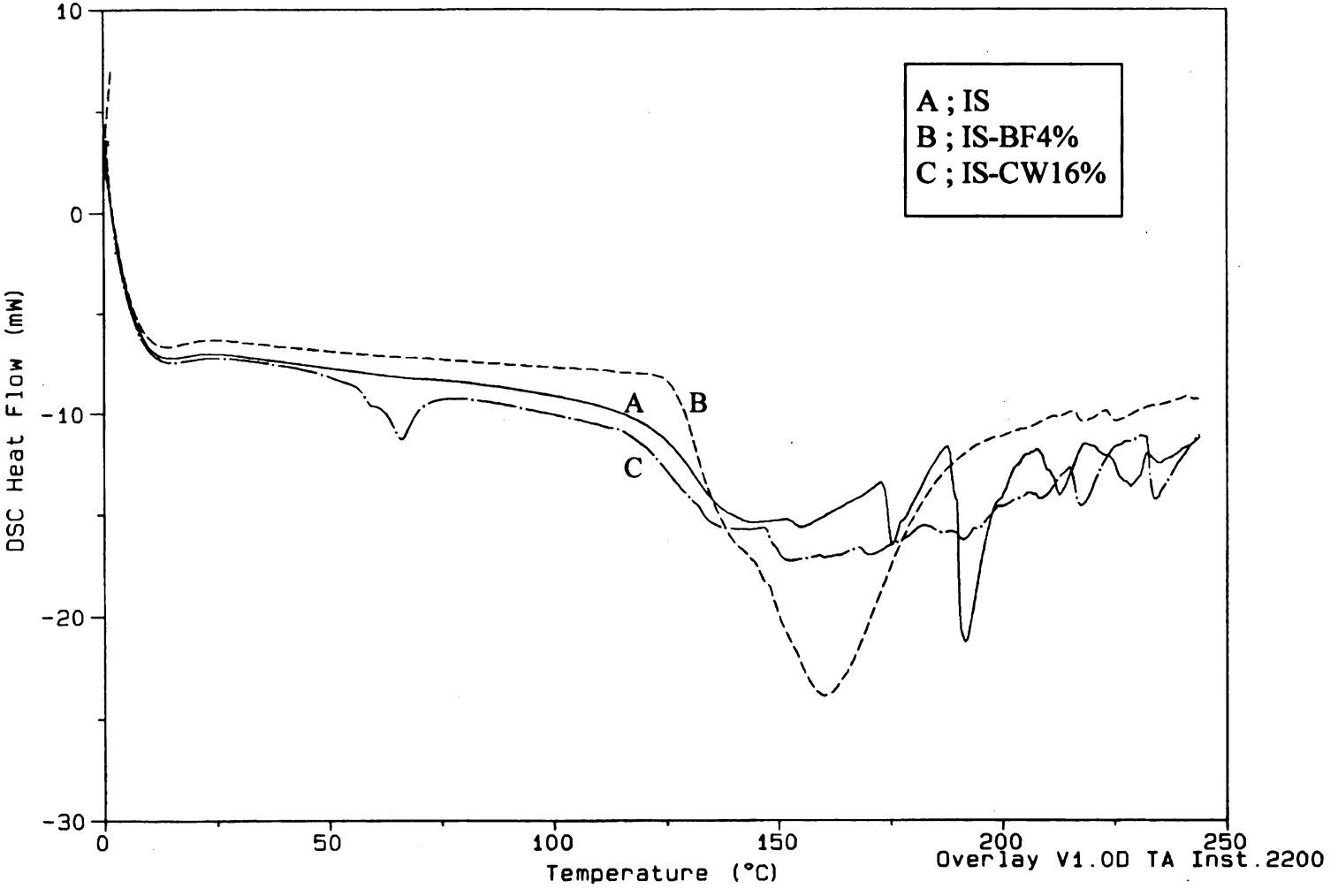


Figure 4.2. Differential Scanning Calorimetry thermogram of films plasticized with sorbitol. (20°C/min). I = whey protein isolate; S = sorbitol; BF = butter fat; CW = candelilla wax.

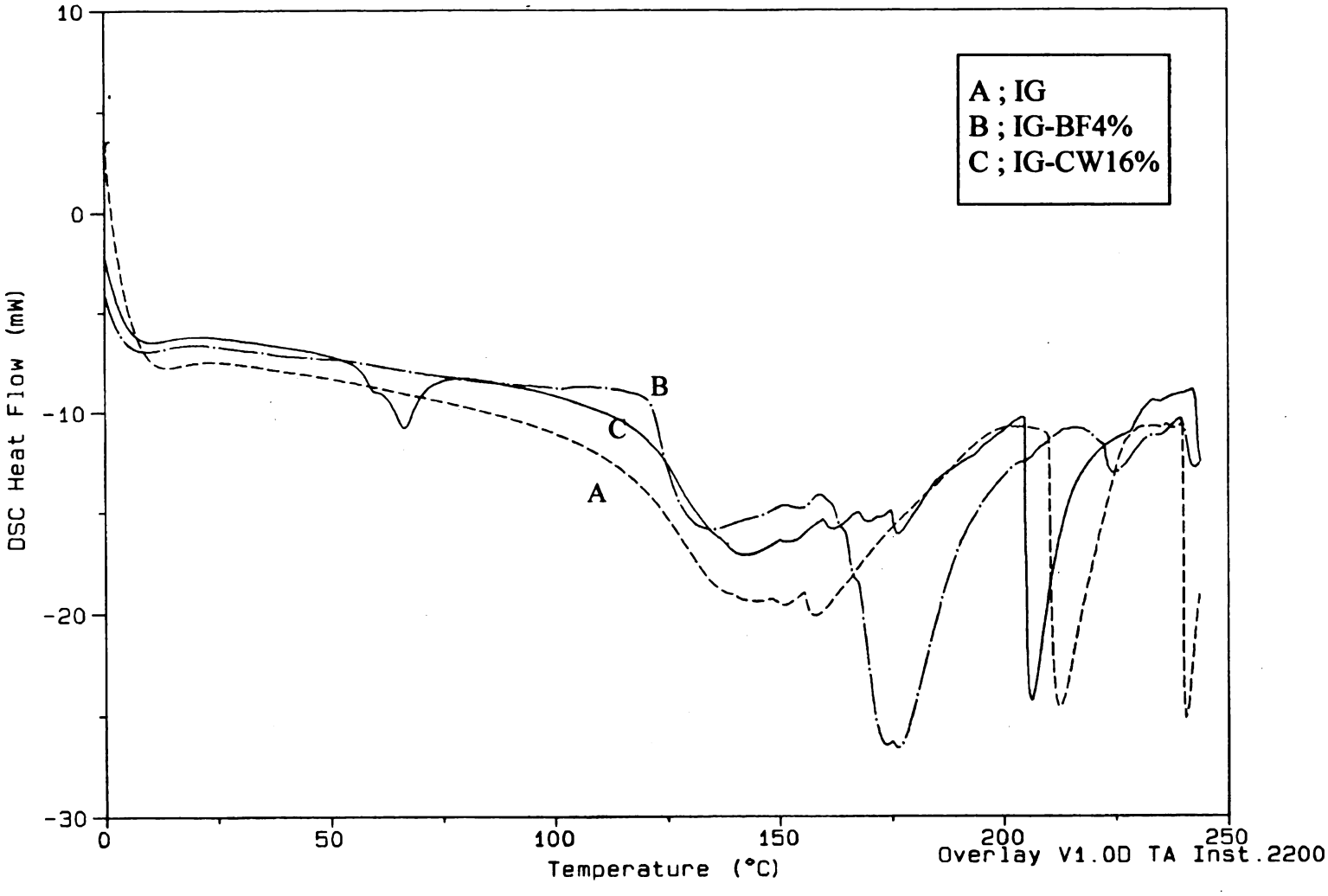


Figure 4.3. Differential Scanning Calorimetry thermogram of films plasticized with glycerol. (20°C/min). I = whey protein isolate; G = glycerol; BF = butter fat; CW = candelilla wax.

Table 4.3. Thermal properties of film forming components as determined by Differential Scanning Calorimetry.

Film components [1]	Transition temperatures (°C) [2]		Heat flows
	T_o	T_p [3]	(J/g)
WPI	125	156	193.2
	215	241	65.0
Sorbitol	96	101	192.2
Glycerol	165	178	66.7
BF	2	10	30.9
	29	30	10.2
	99	101	181.1
CW	57	68	211.2

[1] WPI = whey protein isolate; BF = butter fat; CW = candelilla wax.
[2] T_o = onset transition temperature; T_p = peak transition temperature.
[3] Only the first peak temperature was reported if more than two peaks were observed.

Table 4.4. Thermal properties of whey protein/lipid emulsion edible films as determined by Differential Scanning Calorimetry [1].

Treatments [1]	Transition temperatures (°C) [2]		Heat flows
	T_o	T_p [3]	(J/g)
IS	126	143	15.6
IS-BF4%	127	160	202.9
IS-CW16%	60	66	5.4
	127	135	84.0
IG	108	145	169.9
IG-BF4%	122	132	31.8
IG-CW16%	60	66	7.0
	116	142	208.0

[1] WPI = whey protein isolate; S = sorbitol; BF = butter fat; CW = candelilla wax; G = glycerol
[2] T_o = onset transition temperature; T_p = peak transition temperature.
[3] Only the first peak temperature was reported if more than two peaks were observed.

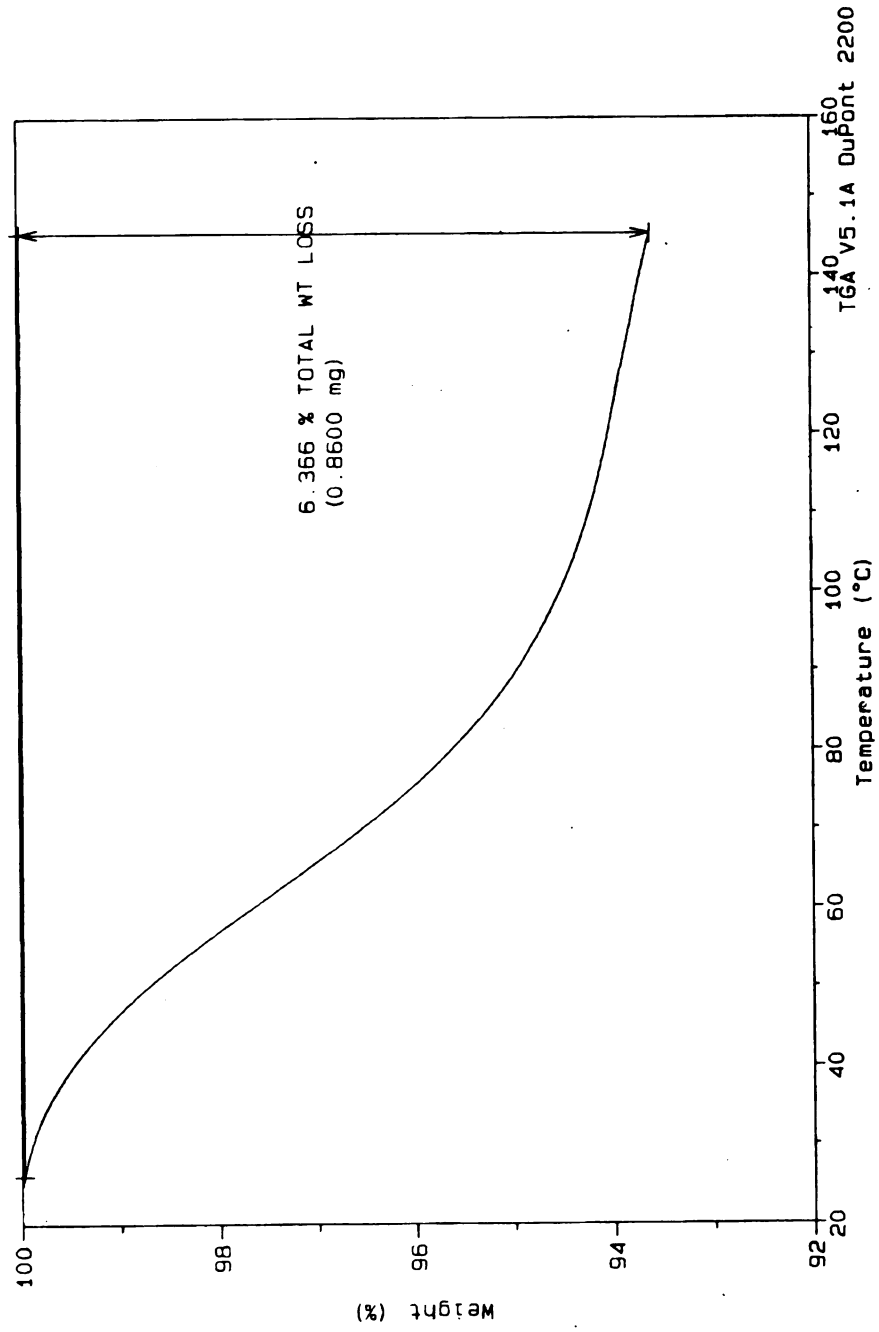


Figure 4.4. Thermogravimetric Analysis thermogram of whey protein isolate powder (20°C/min).

T_o at 125°C and T_p at 156°C with heat flow change of 193.2 J/g (Table 4.3). WPI powder had one more peak at 241°C, suggesting degradation of the protein.

In Figure 4.1 and Table 4.3, sorbitol showed a narrow endothermic peak (96-106°C) as with crystalline polymers. The T_p of sorbitol was 101°C, which was close to the melting temperature of anhydrous sorbitol, 110-112°C (Budavari et al., 1989). Glycerol showed a rather broad endothermic peak (165-220°C) with the heat flow change of 66.65 J/g. Since its melting temperature is 17.8°C, the T_p (178°C) of glycerol may be due to its decomposition, which corresponds to the degradation temperature of 182.2°C at 20mmHg reported by Budavari et al. (1989). BF showed two low T_p at 10 and 30°C, which were within a melting temperature range (0 - 40°C) of BF (Lane, 1992). BF had one more distinctive peak at 101°C, which may correspond to the decomposition of BF. CW showed T_p at 68°C with heat flow change of 211.2 J/g. This temperature was similar to the melting temperature of CW, 64.0°C, reported by Donhowe and Fennema (1993).

All films showed broad endothermic peaks in the temperature range of 108-221°C (Table 4.4). The T_o of glycerol-plasticized WPI films were slightly lower than T_o of sorbitol-plasticized WPI films, 108-122°C and 126-127°C, respectively, probably due to the differences in the plasticizing effect of glycerol and sorbitol. A plasticizer's functional efficacy is often estimated by examining the reduction caused in the glass transition temperature or melting temperature of polymers (Karlsson and Singh, 1998). Glycerol with its lower melting temperature (17.8°C) may be more effective than sorbitol with higher melting temperature (101°C) in lowering thermal transition temperatures (T_o) of whey protein-based films. However, this effect of the plasticizer was not as great as

anticipated. A possible explanation may be the lack of pre-conditioning (i.e., drying) for WPI powder prior to DSC analysis. To confirm this, TGA was used to determine mass changes of WPI powder during heating under conditions close to that of the DSC experiment. Indeed, a 6.4% weight loss was observed (Figure 4.4) due to the moisture in the WPI powder. Water is known to also act as a plasticizer, and any water molecules present in WPI powder could reduce thermal transition temperatures and conformed the effects observed due to the differences in the plasticizer (Pouplin et al., 1999). Further studies are needed with regards to the influence of the plasticizer in thermal transition temperatures of protein-based edible films.

Film samples containing CW showed what appeared to be two endothermic peaks (Figures 4.2, 4.3), and a more definite narrow endothermic peak at 66°C, corresponding to T_p of CW, 68°C (Tables 4.3, 4.4). All films with the exceptions of BF-added films showed multiple peaks around 175-212°C, which appeared to be the degradation temperatures of films (Figures 4.2, 4.3).

4.4.2 Heat sealability and seal strength of the films

Films were heat sealed only on the non-lipids oriented side of the film because lipid-oriented sides did not seal or formed seals that easily delaminated. Heat-sealing was conducted near the onset thermal transition temperatures (T_o) of whey protein/lipid emulsion films. The thermal transition temperature sets the application, such as heat-sealing, temperature range of a polymer (Hernandez, 1997).

The seal strength measurements of sorbitol- and glycerol-plasticized whey protein/ lipid emulsion films are shown in Table 4.5 and Table 4.6, respectively. All films were

Table 4.5. Seal strength (N/m) of whey protein/lipid emulsion edible films plasticized with sorbitol.

Parameter			Treatments [1]		
Temp. (°C)	Pressure (psi)	Dwell time (sec)	IS	IS-BF4%	IS-CW16%
110	40	1	110 ± 16 ^{d}	115 ± 14 ^{d}	105 ± 9 ^{e}
		3	147 ± 9 ^{c}	127 ± 8 ^{d}	119 ± 4 ^{e}
	60	1	116 ± 10 ^{d}	112 ± 19 ^{d}	108 ± 15 ^{e}
		3	158 ± 11 ^{bc}	124 ± 12 ^{d}	120 ± 20 ^{e}
120	40	1	160 ± 15 ^{bc}	150 ± 5 ^{c}	152 ± 8 ^{d}
		3	191 ± 26 ^{b}	191 ± 24 ^{b}	186 ± 12 ^{c}
	60	1	162 ± 10 ^{bc}	150 ± 13 ^{c}	154 ± 5 ^{d}
		3	188 ± 18 ^{b}	178 ± 8 ^{b}	184 ± 21 ^{c}
130	40	1	293 ± 12 ^{a}	215 ± 13 ^{ab}	248 ± 17 ^{b}
		3	298 ± 28 ^{a}	268 ± 16 ^{a}	285 ± 15 ^{a}
	60	1	284 ± 28 ^{a}	239 ± 17 ^{a}	236 ± 11 ^{b}
		3	301 ± 19 ^{a}	261 ± 29 ^{a}	296 ± 6 ^{a}

$^{a\text{-}fe}$ Different letters columnwise denotes significant difference ($p < 0.05$), n = 3 for all treatments.
[1] I = whey protein isolate; S = sorbitol; BF = butter fat; CW = candelilla wax

Table 4.6. Seal strength (N/m) of whey protein/lipid emulsion edible films plasticized with glycerol.

Parameter			Treatments [1]		
Temp. (°C)	Pressure (psi)	Dwell time (sec)	IG	IG-BF4%	IG-CW16%
110	40	1	285 ± 23 [a]	265 ± 9 [a]	261 ± 10 [b]
		3	323 ± 42 [a]	288 ± 19 [a]	297 ± 15 [a]
	60	1	282 ± 10 [a]	275 ± 40 [a]	263 ± 8 [b]
		3	296 ± 37 [a]	291 ± 38 [a]	291 ± 17 [a]
120	40	1	211 ± 17 [c]	216 ± 13 [b]	213 ± 21 [c]
		3	257 ± 12 [b]	269 ± 25 [a]	260 ± 20 [b]
	60	1	225 ± 8 [c]	217 ± 11 [b]	202 ± 16 [cd]
		3	263 ± 28 [ab]	262 ± 16 [a]	257 ± 18 [b]
130	40	1	171 ± 20 [d]	147 ± 57 [c]	141 ± 36 [d]
		3	187 ± 22 [d]	169 ± 10 [c]	173 ± 9 [d]
	60	1	173 ± 5 [d]	159 ± 28 [c]	156 ± 21 [d]
		3	198 ± 15 [cd]	165 ± 21 [c]	168 ± 27 [d]

[a-d] Different letters columnwise denotes significant difference ($p < 0.05$), n = 3 for all treatments.
[1] I = whey protein isolate; G = glycerol; BF = butter fat; CW = candelilla wax

Table 4.7. Seal strength (N/m) of various edible films and synthetic polymers.

Films [1]	Thickness (μm)	Heat-sealing conditions (temp., pressure, dwell time)	Seal strength (N/ m)	References
IS-CW	140	130°C, 60psi, 3sec	301	Present study
IG-CW	120	110°C, 40psi, 3sec	323	Present study
LS-CW	110	120°C, 40psi, 4sec	247	Chick, 1998
LS-CW	110	107°C, 40psi, 4sec	153	Chick, 1998
CG	50	NA[2]	137	Ninomiya et al., 1990
CS	50	NA	130	Ninomiya et al., 1990
PVDC-PP/ LDPE	24/ 50	NA	1737	Martin, 1986.
Nylon/ LDPE	11/ 50	NA	1544	Martin, 1986.
PET/ LDPE	12/ 38	NA	733	Martin, 1986.

[1] IS-CW = whey protein isolate (50%) - sorbitol (42%) - candelilla wax (8%);
IG-CW = whey protein isolate (60%) - glycerol (30%) - candelilla wax (10%);
LS-CW = lactic acid casein (50%) - sorbitol (35%) - candelilla wax (15%);
CG = carageenan (70%) - glycerol (30%); CS = carageenan (50%) - sorbitol (50%);
PVDC = polyvinyl chloride; PP = polypropylene; LDPE = low density polyethylene; PET = polyethylene terephthalate.
[2] NA = not available.

heat sealable. The seal strength of sorbitol-plasticized films ranged from 105 to 301 N/m, while glycerol-plasticized films showed seal strength between 141 to 323 N/m. Overall, the seal pressure and dwell time variation did not produce significant differences in the seal strengths of the films. However, heat-sealing temperature significantly ($p < 0.05$) influenced seal strength of the films. The highest seal strengths ($p < 0.05$) were observed at temperature 130°C for sorbitol-plasticized WPI films (Table 4.5), and 110°C for glycerol-plasticized WPI films (Table 4.6). These heat-sealing temperatures corresponded with the T_o of the films from DSC analysis (Table 4.4). Sorbitol-plasticized WPI films had a T_o of 126-127°C, and optimum seal strength was obtained when films were sealed at 130°C. In case of glycerol-plasticized films, they had a T_o of 108-122°C, and optimum seal strength was obtained at 110°C. These results indicate that the T_o may be used to determine thermal processing temperatures for protein-based edible films.

Lower seal strength of glycerol-plasticized films at 130°C may be due to the excessive temperature resulting in distorted and weakened seals. According to Martin (1986), when the heat required to produce a seal exceeds the heat-sealing temperature range for that material, it induces a distorted or nonfunctional seal. In our study, slight deformation of seal structure was observed with glycerol-plasticized films at 130°C indicating that degradation of the materials started to occur at this temperature, thus, reducing seal strength. It is recommended that heat-sealing temperature should not exceed 130°C for glycerol-plasticized WPI films.

The highest seal strength obtained with sorbitol- and glycerol-plasticized films were 301 and 323 N/m, respectively. These results were lower than the seal strengths of most synthetic polymers, but comparable to the seal strength obtained with lactic acid

casein- or carageenan-based edible films (Table 4.7). However, these are very general comparisons, as stated previously caution must be taken in comparing data of this nature since different experiments were conducted under different heat-sealing and testing conditions.

4.4.3 Surface elemental compositions of the films determined by ESCA survey spectra.

Figures 4.5 and 4.6 show survey spectra of sorbitol- or glycerol-plasticized WPI films containing BF or CW before and after heat-sealing. Surface elemental compositions of the films were calculated and are shown in Table 4.8. All treatments showed distinctive peaks in O1s, N1s and C1s regions except no N1s spectra were observed for the unsealed sorbitol-plasticized films containing BF and CW. The absence of N1s spectra is probably due to low N ratio and/or poor quality of the spectra. Thus it was difficult to determine relative changes of elemental composition upon heat-sealing of the sorbitol-plasticized WPI films containing BF and CW. Except for these two films mentioned, the other films (sorbitol-plasticized WPI films, glycerol-plasticized WPI films, and glycerol-plasticized films containing BF and CW) showed distinctive changes in their surface elemental compositions upon heat-sealing.

Carbon was the main element of the films, and oxygen was the second most prominent element. Nitrogen was less than 8.3% in all films. Carbon comprised 71.5-77.4% of the total surface composition of the unsealed films. After heat-sealing, carbon compositions declined approximately 1.4-6.5% depending on the film. Oxygen and nitrogen compositions, on the other hand, increased upon heat-sealing. Approximately 1-

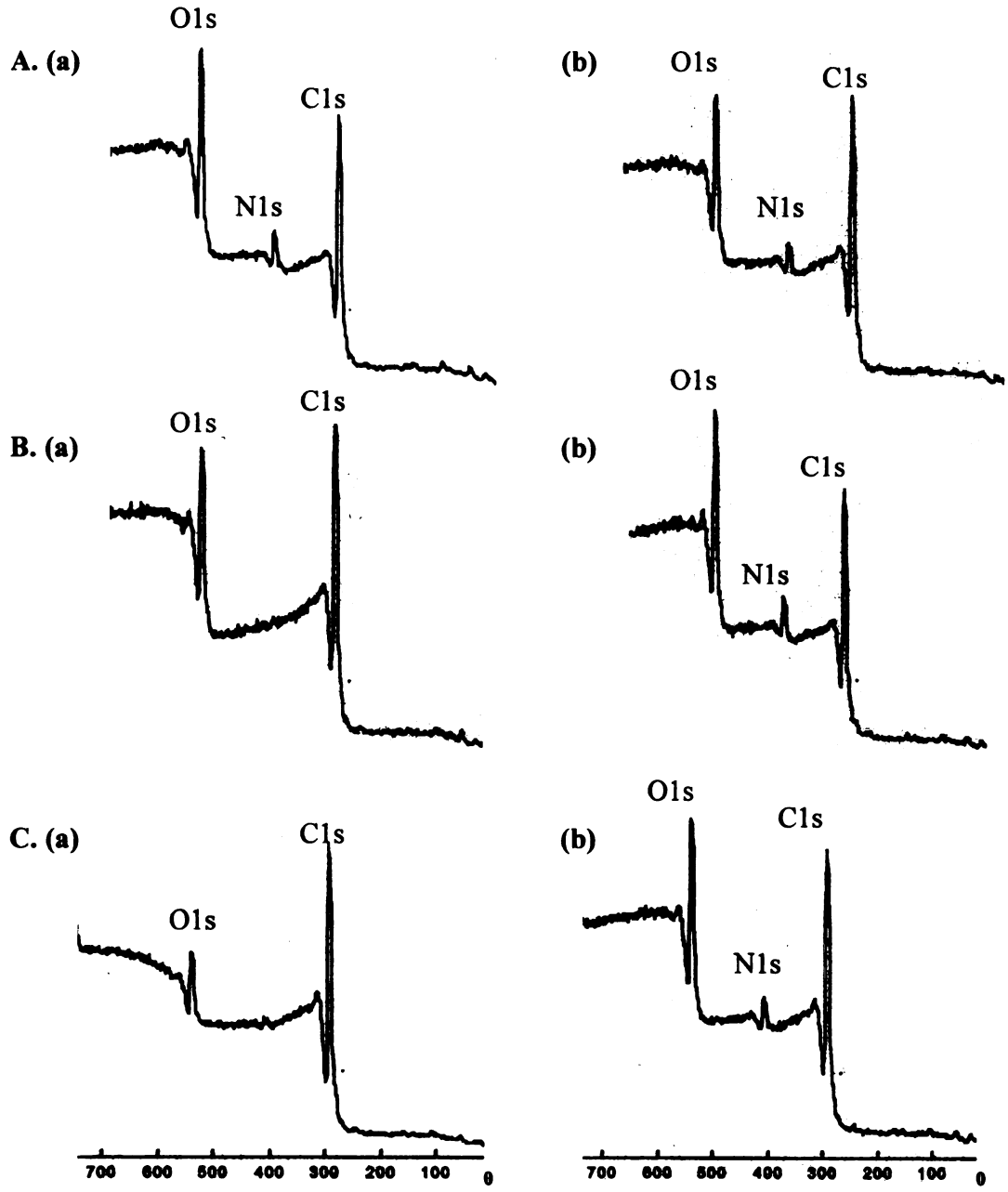


Figure 4.5. Electron Spectroscopy for Chemical Analysis survey spectra of whey protein/lipid emulsion edible films plasticized with sorbitol.

A. (a) IS film unsealed, (b) IS film sealed ;

B. (a) IS-BF4% film unsealed, (b) IS-BF4% film sealed ;

C. (a) IS-CW16% film unsealed, (b) IS-CW16% film sealed

I = whey protein isolate; S = sorbitol; BF = butter fat; CW = candelilla wax

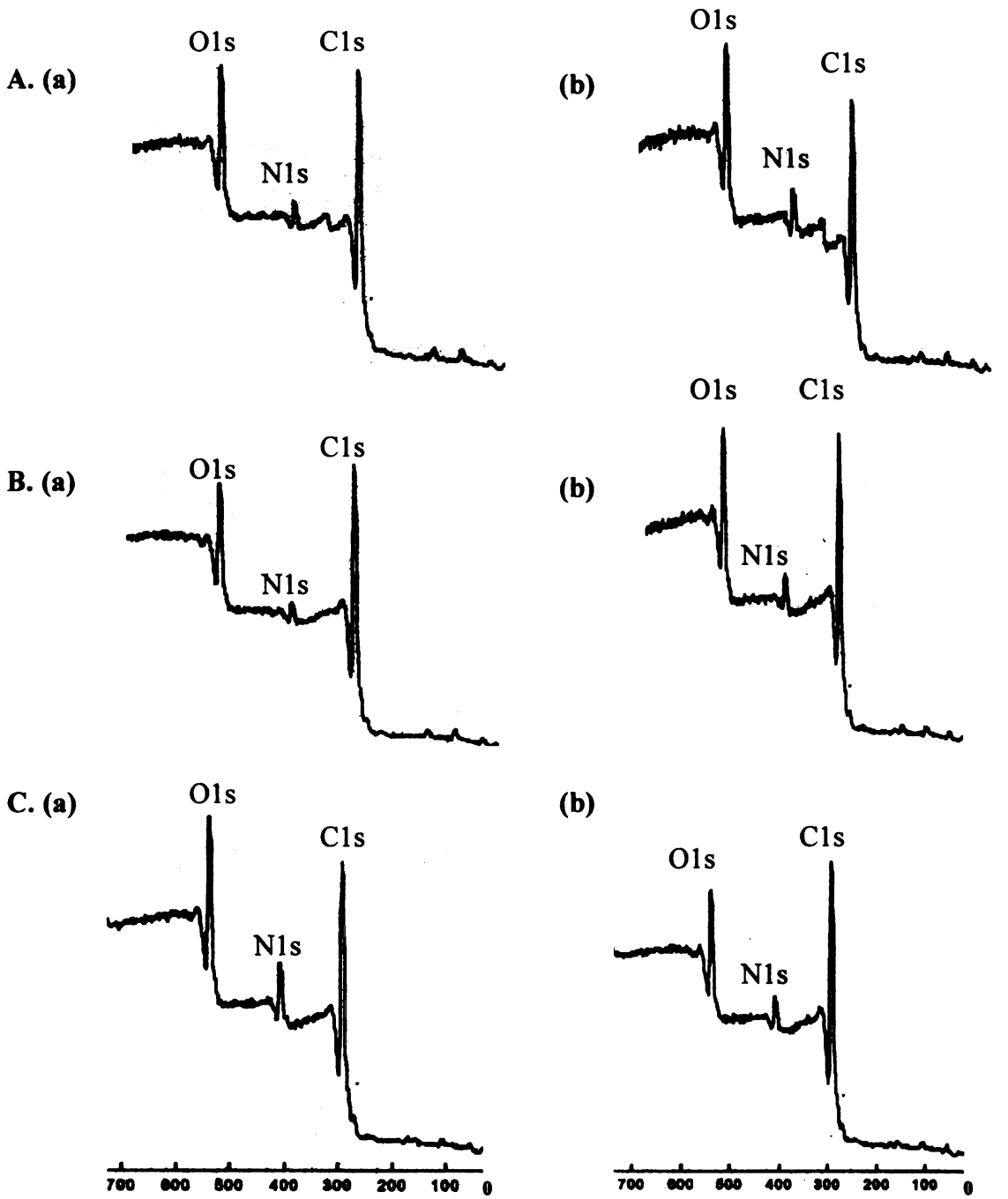


Figure 4.6. Electron Spectroscopy for Chemical Analysis survey spectra of whey protein/lipid emulsion edible films plasticized with glycerol.

A. (a) IG film unsealed, (b) IG film sealed ;

B. (a) IG-BF4% film unsealed, (b) IG-BF4% film sealed ;

C. (a) IG-CW16% film unsealed, (b) IG-CW16% film sealed

I = whey protein isolate; G = glycerol; BF = butter fat; CW = candelilla wax

Table 4.8. Surface elemental compositions of whey protein/lipid emulsion edible films before and after heat-sealing determined with survey spectra of Electron Spectroscopy for Chemical Analysis.

	% C		% O		% N	
Treatments[1]	unsealed	sealed	unsealed	sealed	unsealed	sealed
IS	71.5	68.9	24.4	25.8	4.0	5.3
IS-BF4%	66.5	66.9	33.3	25.6	0.2	7.5
IS-CW16%	59.6	72.7	40.4	21.4	0.0	5.9
IG	77.4	70.9	18.1	20.8	4.5	8.3
IG-BF4%	76.7	73.1	18.1	20.9	5.1	6.0
IG-CW16%	76.5	70.8	18.2	21.3	5.3	7.9

[1] I = whey protein isolate; S = sorbitol; G = glycerol; BF = butter fat; CW = candelilla wax

2% increases occurred for the oxygen components on the surfaces of the sealed films, whereas nitrogen increased by 1-4% again depending on the film (Table 4.8), indicating that some oxygen and nitrogen components have formed on the surfaces of sealed films upon heat-sealing.

4.4.4 Surface compositional changes of the films upon heat-sealing

To further understand the mechanism of sealing, high resolution ESCA peaks were obtained from the unsealed and sealed WPI films. Figure 4.7 showed the high-resolution C1s, O1s and N1s spectra of the sorbitol-plasticized WPI films before and after heat-sealing. Since all films showed similar spectra, only this spectrum (Figure 4.7) was selected to be presented here. Each peak was assigned according to their binding energy (eV). The assignments were made by comparing observed binding energies to comparable data from the literature (Briggs and Seah, 1990; Buchwalter, 1995; Ratner and Castner, 1997; Pleul et al., 1998). Peak positions and assignments are given in Table 4.9. Surface compositions of the films before and after heat-sealing are presented in Tables 4.10, 4.11 and 4.12.

The C1s spectra (Figures 4.7a, d) was composed of four components and the components appeared at 284.6eV (C-H), 286.3eV (C-O), 287.6eV (O=C) and 289.5eV (O-C=O; Table 4.9). The relative concentrations of the components are listed in Table 4.10. Main components of C1s spectra appeared to be C-H and C-O. A comparison of the C1s spectra for all unsealed and sealed films indicated that the relative intensities of different carbon components were essentially the same for the unsealed and the sealed surfaces of the films.

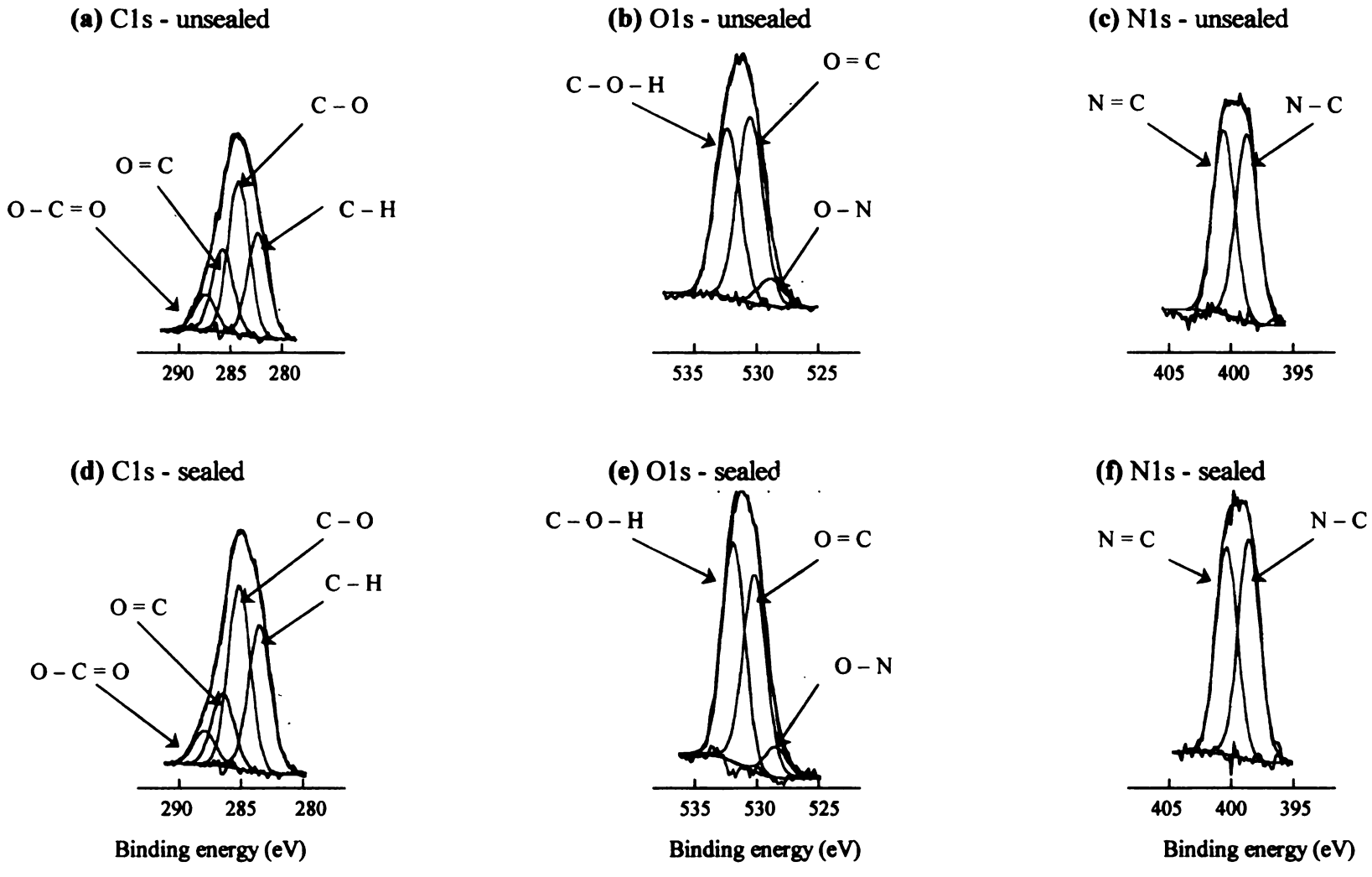


Figure 4.7. Representative Electron Spectroscopy for Chemical Analysis spectra of unsealed and sealed sorbitol-plasticized whey protein isolate films. unsealed (a) C1s; (b) O1s; (c) N1s, sealed (d) C1s; (e) O1s; (f) N1s.

Table 4.9. Binding energies of peaks in the Electron Spectroscopy for Chemical Analysis spectra of whey protein/lipid emulsion edible films.

	C1s			
Peaks	C - H (eV)	C - O (eV)	O = C (eV)	O - C = O (eV)
Binding energy	284.6	286.3	287.6	289.5

	O1s			N1s	
Peaks	O - N (eV)	O = C (eV)	C - O - H (eV)	N - C (eV)	N = C (eV)
Binding energy	530.4	532.3	534.3	399.7	401.8

Table 4.10. Surface compositions of whey protein/lipid emulsion edible films before and after heat sealing as determined by the Electron Spectroscopy for Chemical Analysis spectra C1s.

	C1s							
	C - H (%)		C - O (%)		O = C (%)		O - C = O (%)	
Treatments[1]	Unsealed	Sealed	Unsealed	Sealed	Unsealed	Sealed	Unsealed	Sealed
IS	27.8	35.1	40.7	41.0	22.0	16.3	9.5	7.6
IS-BF4%	32.0	26.7	35.0	40.0	22.8	24.9	4.7	8.4
IS-CW16%	40.3	38.9	32.6	26.9	22.4	30.8	0.0	3.4
IG	31.7	34.0	32.9	43.2	28.9	16.6	6.6	6.2
IG-BF4%	33.7	36.6	34.7	35.4	26.2	20.7	5.5	7.6
IG-CW16%	31.9	33.9	38.1	27.4	22.3	31.4	7.8	7.3

[1] I = whey protein isolate; S = sorbitol; G = glycerol; BF = butter fat; CW = candelilla wax

Table 4.11. Surface compositions of whey protein/lipid emulsion edible films before and after heat sealing as determined by Electron Spectroscopy for Chemical Analysis spectra O1s.

	O1s					
	O - N (%)		O = C (%)		C - O - H (%)	
Treatments[1]	Unsealed	Sealed	Unsealed	Sealed	Unsealed	Sealed
IS	7.2	7.3	48.7	44.9	44.1	47.8
IS-BF4%	11.4	4.1	47.9	46.4	40.7	49.5
IS-CW16%	11.2	8.0	44.8	44.6	43.8	47.4
IG	10.9	8.6	47.8	47.1	41.3	44.2
IG-BF4%	12.5	8.1	46.3	46.2	41.2	45.7
IG-CW16%	12.7	12.0	48.5	46.8	38.8	43.2

[1] I = whey protein isolate; S = sorbitol; G = glycerol; BF = butter fat; CW = candelilla wax.

Table 4.12. Surface compositions of whey protein/lipid emulsion edible films before and after heat sealing as determined by Electron Spectroscopy for Chemical Analysis spectra N1s.

	N1s			
	N - C (%)		N = C (%)	
Treatments[1]	Unsealed	Sealed	Unsealed	Sealed
IS	50.5	52.5	49.5	47.6
IS-BF4%	ND	46.8	ND	53.3
IS-CW16%	ND	51.7	ND	48.3
IG	49.9	55.3	50.1	44.7
IG-BF4%	54.4	56.3	45.6	43.8
IG-CW16%	53.0	53.5	47.0	46.5

[1] I = whey protein isolate; S = sorbitol; G = glycerol; BF = butter fat; CW = candelilla wax; ND = not detectable

In Figures 4.7b and 4.7e, the O1s spectra of the sorbitol-plasticized WPI films showed three components, C-O-H at 534.3eV, O=C at 532.3eV and O-N at 530.4eV (Table 4.9). The relative concentrations of these components for unsealed and sealed films are shown in Table 4.11. Main components of O1s spectra were O=C and C-O-H. Overall, O=C percentage was higher for the unsealed films and C-O-H amount was higher in the sealed films. In Figures 4.7b and 4.7e, shifting of O=C to C-O-H was observed indicating disappearance of O=C component and formation of C-O-H component upon heat-sealing. Formation of C-O-H may be responsible for the sealing of whey protein-based films.

The N1s spectra for the unsealed and sealed films appeared at 399.7eV (N-C) and 401.8eV (N=C; Figures 4.7c, f). Similar trends were observed as with the results of O1s spectra. The relative intensity of the N=C component was higher in the unsealed sorbitol-plasticized WPI films, while the N-C intensity was higher in the sealed films (Figures 4.7c, f). Similar trends were observed with other films (Table 4.12). Based on the spectra intensity changes, it may be concluded that C-O-H (534.3eV) and N-C (399.7eV) components may be responsible for heat-sealing mechanism of whey protein-based films.

Wu et al. (1995) employed ESCA to investigate adhesive bondings on the surface of ammonia treated polyolefins. Reportedly, C-O-H components on the surfaces participated in hydrogen bonding formation across the interfaces of polyolefins, also amine groups on the surfaces formed covalent bonds at the interface. In the adhesion mechanism of polymers, hydrogen bonds and covalent bond are known to play critical roles in interfacial interactions to achieve sufficient bond strength (Allen, 1987; Urban, 1993; Misra, 1994).

In proteins, hydrogen bonds may appear between oxygen of the C=O (carbonyl groups: hydrogen acceptors) of a peptide bond and hydrogen of -NH, or -OH (imino groups, or hydroxyl groups: hydrogen donor) of another peptide bond (Cheftel et al., 1985):

$$C = O \bullet\bullet H - N$$

Hydrogen bonds are one of the main forces that are involved in structural formation of protein-based films. Plasticizers are typically added to increase flexibility of protein film, which also provides for additional hydrogen bonding. For example, upon addition of glycerol as a plasticizer:

$$\text{H-O (Glycerol)} + C = O \bullet\bullet H - N \rightarrow C = O \bullet\bullet \text{H-O (Glycerol)} + H - N$$

Plasticizers, such as glycerol, have been reported to act as heat-sealing promoters (Georgevits, 1967). Figure 4.8 provides proposed models for nature of hydrogen bonding between plasticizer and plasticizer, plasticizer and protein, and protein and protein upon heat-sealing of whey protein-based films. It is assumed that glycerol and sorbitol will behave in a similar manner with regards to hydrogen bonding. Therefore, only one example is provided with glycerol and one with sorbitol in Figure 4.8.

β-Lg is the major whey protein, and aspartic acid (11%) and glutamic acid (16%) are the most abundant amino acids in β-Lg. These two amino acids are also abundant in α-La, 9 and 8%, respectively (Appendix I). Carboxyl groups in aspartic acid and glutamic acid residues may be involved in the formation of hydrogen bonds (Figure 4.8). Another major amino acid both in β-Lg (15%) and α-La (12%) is lysine (Appendix I). Lysine has a reactive ε-NH_2 group that is available for covalent bond formation with glutamine or asparagine amino acid residues, upon heat treatments (Cheftel et al., 1985). A proposed model for these covalent bond formations on the surface of heat sealed whey protein-

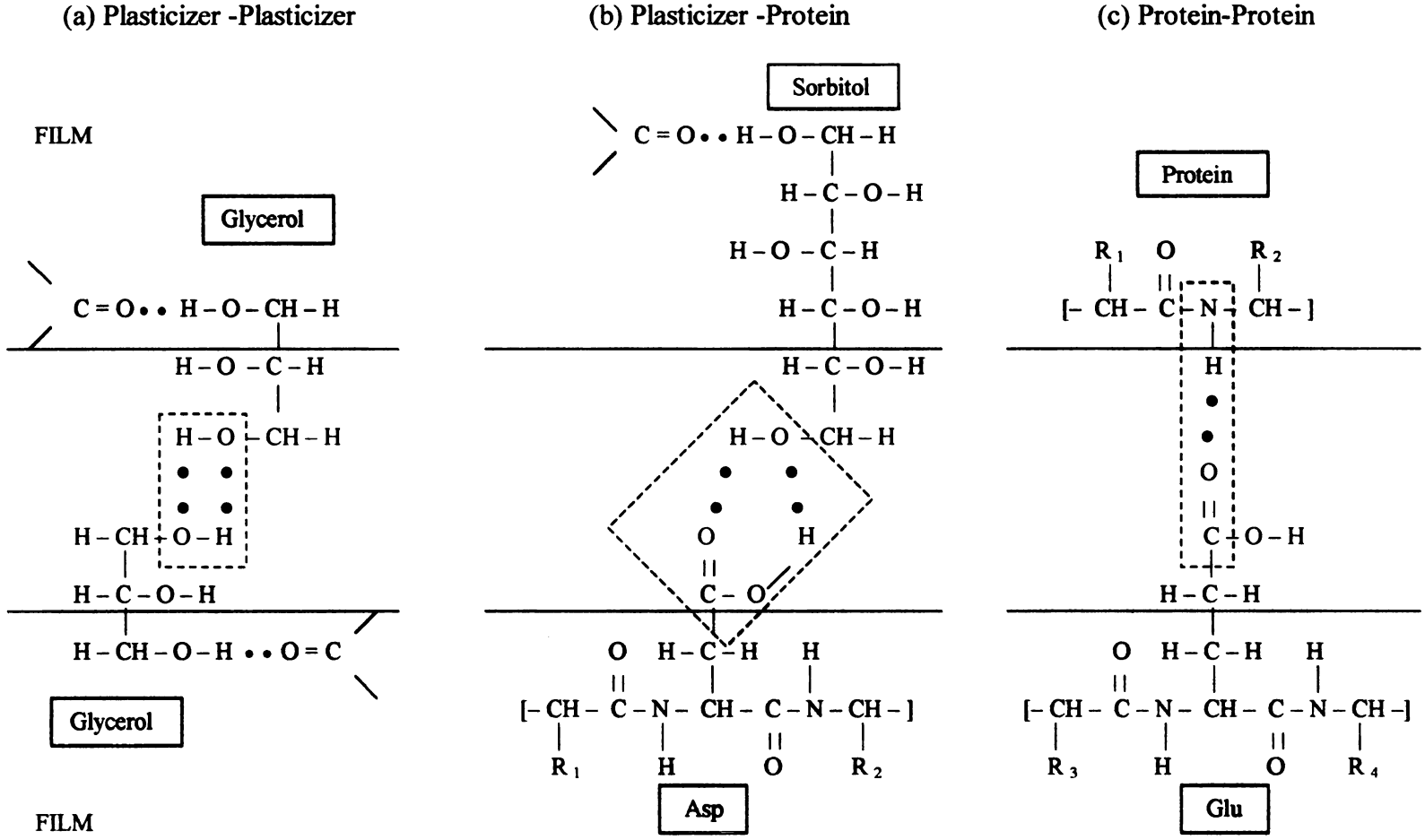


Figure 4.8. Proposed model for hydrogen bonding between (a) plasticizer-plasticizer, (b) plasticizer-protein, (c) protein-protein upon heat-sealing of glycerol- or sorbitol-plasticized whey protein-based edible films.

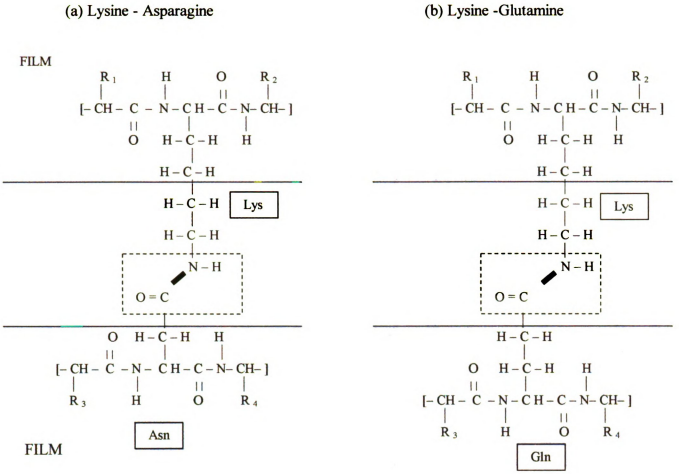


Figure 4.9. Proposed model for covalent bonding between (a) lysine-asparagine, (b) lysine-glutamine upon heat-sealing of whey protein-based edible films.

based films are illustrated in Figure 4.9. These proposed models in Figure 4.8 and 4.9 would account for the increases in C-O-H and N-C observed with ESCA due to heat-sealing of the whey protein-based films. Although not illustrated in this study, other possible interfacial bondings include van der Waals forces and electrostatic interaction (Urban, 1993).

In summary, thermal analysis of whey protein/lipid emulsion films was an efficient tool to obtain information with regards to heat processing, i.e., heat-sealing and degradation of the films. All films were heat sealable and showed good seal strength that were comparable to the seal strength of lactic acid casein- and carageenan-based edible polymers. Optimum heat-sealing temperatures (that provided the highest seal strength) were 130 and 110°C for sorbitol- and glycerol-plasticized films, respectively. Formation of C-O-H and N-C bonds appeared to be important in obtaining a sealed joint for the whey protein-based edible films.

CHAPTER 5

SUITABILITY OF WHEY PROTEIN/ LIPID EMULSION EDIBLE FILMS AS FOOD PACKAGING MATERIALS.

5.1 ABSTRACT

Whey protein isolate (WPI; 5%, w/v) films plasticized with sorbitol (4.2-5%, w/v) or glycerol (2.7-3.5%, w/v) were prepared with butter fat (BF; 4%, w/w of protein) or candelilla wax (CW; 0.8%, w/v) to produce whey protein/lipid emulsion edible films. Moisture contents, water solubilities, moisture sorption isotherm, and sensory attributes of the films were evaluated to determine their suitability as food packaging materials. Heat sealed edible pouches were manufactured using the glycerol-plasticized WPI films containing CW16%. The pouches were used to package and store powder cocoa mix. Results of these studies showed that solubility of the films in water were affected by plasticizer type; the higher the plasticizer amount, the greater was the solubility. Moisture contents of the films were also influenced by plasticizer type. Lower moisture content of the films resulted in lower equilibrium moisture contents at all a_w levels. Sensory evaluation of the films revealed that WPI films had no distinctive milk odor. However, the films were perceived to be slightly sweet and adhesive by the trained panelists. Results from the storage study with powder cocoa mix packaged in edible whey protein-based pouches showed that these films were suitable for packaging powder cocoa mix and may be suitable for packaging of non-hygroscopic foods.

5.2 INTRODUCTION

Various applications for free-standing protein-based edible films have been proposed (Debeaufort et al., 1998). These proposed applications include packaging individual portions of foods using edible films, dividing components within one food using these films, and soluble packages for pre-measure food ingredients or additives. However, so far not many of these proposed applications have been investigated.

Water solubility, moisture sorption isotherm, sensory attributes and stability during storage are all important attributes when considering protein-based films as packaging materials. Edible films with high water solubility may be required for a pouch packaging containing pre-measured portions which should be released into the water quickly. Also, instant dried food preparation (as with individual beverage mixes or soups) requires quick dispersion, thus very soluble materials (Gontard and Guilbert, 1994). Conversely, the insolubility of edible films needs to be considered for other specific applications, such as when the film has to be in contact with water during processing without releasing its contents or if controlled release is desirable (Gontard et al., 1994).

Typically, protein-based films are sensitive to humidity changes (Frederick, 1996). Moisture migration in food can induce adverse effects on the stability of food products. The microbial and physical stability, sensory quality, and enzymatic reaction in foods are greatly influenced by moisture content, which can change drastically by the loss or gain of moisture during processing and/or storage (Fennema, 1985). Thus, water sorption is of practical interest when considering particular materials for food packaging use.

Sensory attributes of edible films, on the other hand, are important for consumer acceptance. Food preferences by human often are based on sensory attributes, such as

appearance, color, flavor, texture and mouth feel (Damodaran, 1996). When consumed with other food products, it is desirable that edible films be as tasteless as possible in consideration for consumer acceptance (Gontard and Guilbert, 1994). There is very little published on the sensory properties of edible films. So far no study has been reported on the sensory attributes of whey protein-based films.

We have developed whey protein/lipid emulsion films containing butter fat or candelilla wax, and reported their barrier and mechanical properties in chapter 2. Also, we reported on the thermal properties, heat sealability and seal strength of these films in chapter 4. The significance of research of this nature is to demonstrate its possible application. Thus, the intent of this particular study was to determine moisture contents, solubilities in water, moisture sorption properties and sensory attributes of whey protein/lipid emulsion films to establish their suitability as food packaging materials. The films' performances as pouch packaging materials were investigated for powder cocoa mix.

5.3 MATERIALS AND METHODS

5.3.1 Materials

Sodium azide was purchased from Sigma Chemical Co. (St. Louis, MO). For the determination of moisture sorption isotherm of the films, $KC_2H_3O_2$ and $NaNO_2$ were obtained from J.T. Baker Co. (Phillipsburg, NJ). NaCl and KCl were supplied by Mallinckrodt Specialty Chemical Co. (Paris, KY). $LiCl{\cdot}H_2O$ and $K_2CO_3{\cdot}2H_2O$ were purchased from Sigma Chemical Co. (St. Louis, MO), and $CaCl_2{\cdot}2H_2O$ was from Fisher

Scientific Co. (Fair Lawn, NJ). $Mg(NO_3)_2 \cdot 6H_2O$ was provided by EM Science (Cherry Hill, NJ).

For the sensory evaluation, whey protein-based films were prepared with all food grade ingredients. D-Sorbitol and glycerol were donated by Lonza, Inc. (Fair Lawn, NJ). NaOH was purchased from Mallinckrodt Specialty Chemical Co. and was also food grade. Powder cocoa mix was obtained from Nestlé, Inc. (Glendale, CA)

5.3.2 Film preparation

Whey protein/lipid emulsion films were prepared as described in section 2.3.2 of chapter 2 (Figure 2.1). Sorbitol- or glycerol-plasticized whey protein isolate (WPI) films were prepared without, or with butter fat (BF; 4%, w/w of protein) or candelilla wax (CW; 16%, w/w of protein). Compositions of the films produced are shown in Table 3.2 of chapter 3. All food grade ingredients were used to prepare the films used for the sensory evaluation. BF-added films were excluded from sensory evaluation due to their poor acceptability (they appeared and felt greasy) based on the preliminary test results.

5.3.3 Moisture content of the films

Aluminum dishes were weighed and approximately 3 grams of the film was added to each dish. All film samples were weighed to the nearest 0.0001gram before and after drying. Samples were dried in a drying oven (Precision Scientific model 524, Chicago, IL) at 100 ± 2°C for 24 hr. After drying, samples were cooled in desiccator for 30 min to equilibrate to room temperature then re-weighed. Moisture contents of the films were calculated as follows:

$$\text{Moisture (\%)} = [\ (Wi - Wf) / Wi\ ] \times 100 \qquad (20)$$

where, Wi = initial wt. of sample

Wf = final wt. of sample after drying

5.3.4 Solubility of the films in water

A method modified from Gontard et.al. (1992) was used to measure film's water solubility. The water solubility was reported as the percentage of soluble matter to initial dry matter of the film. Approximately 3grams, of a all film sample was weighed to the nearest 0.0001gram before and after drying. The film was weighed and dried in a drying oven (100 ± 2°C, 24 hr) to determine its initial dry matter weight. Another 3grams of film was immersed into 50 ml of water containing trace of sodium azide (0.02 % w/v; to prevent microbial growth), and gently agitated for 24 hr at 20 ± 2°C. Undissolved film was then taken out and dried (100 ± 2°C for 24hr) to determine the weight of dry matter which was not solubilized in water. The weight of dry matter solubilized was calculated by subtracting the weight of dry matter not solubilized from the weight of initial dry matter and was reported on an initial dry weight basis as follows:

$$\text{Water solubility (\%)} = \frac{(\text{wt. of initial dry matter} - \text{wt. of dry matter not solubilized})}{\text{wt. of initial dry matter}} \times 100 \qquad (21)$$

5.3.5 Moisture sorption isotherm of the films

All films were conditioned at 25 ± 0.1°C for 48 hr in hermetically sealed glass jars containing desiccant. First, initial moisture content (IMC) of films were determined.

Aluminum dishes were weighed and approximately 3 grams of the film was added to each dish. All film samples were weighed to the nearest 0.0001gram before and after drying. Samples were dried in a drying oven at 100 ± 2°C for 24 hr. After drying, samples were cooled in desiccator for 30 min to equilibrate to room temperature then weighed to determine weight loss of the samples due to moisture loss. IMC was determined as the percentage of moisture based on the oven dry weight using the following equation:

$$IMC\ (\%) = [(Wi - Wf) / Wf] \times 100 \qquad (22)$$

where, Wi = initial wt. of sample

Wf = final wt. of sample after drying

To determine moisture sorption isotherm (MSI) of samples, temperature was set at 25 ± 0.1°C. Different humidity conditions were prepared by setting saturated salt solution in desiccators. Saturated salt solutions were obtained according to ASTM standard E104 "*Standard practice for maintaining constant relative humidity by means of aqueous solutions*" (ASTM, 1997). Eight different humidity conditions 18 ± 0.5%, 23± 0.5%, 34± 0.5%, 46± 0.5%, 54± 0.5%, 64± 0.5%, 73± 0.5%, and 90± 0.5% were obtained by using the following chemicals: $LiCl{\cdot}H_2O$, $KC_2H_3O_2$, $CaCl_2{\cdot}2H_2O$, $K_2CO_3{\cdot}2H_2O$, $Mg(NO_3)_2{\cdot}6H_2O$, $NaNO_2$, NaCl, and KCl. Aluminum dishes were first weighed; approximately 3grams of the sample was added to each dish. The dishes were then placed in the desiccator, and allowed to equilibrate. The samples were weighed every 2 days until they reached a constant mass (taken as a smaller than 1% change in the mass of the sample). Equilibrium moisture contents (EMC) were calculated as following:

$$EMC\ (\%) = [\{Pf\,(1 + IMC) / Pi\} - 1] \times 100 \qquad (23)$$

where, IMC = gH_2O/g dry wt. product

Pf = final wt. of sample

Pi = initial wt. of sample

The Guggenheim-Anderson-de Boer (GAB) equation was used to fit the moisture sorption data.

5.3.6 Sensory evaluation of the films

Sensory evaluation was conducted using a 15-member trained sensory panel consisting of faculty and graduate students (8 female, 7 male, age 20-55) at Michigan State University. They were selected through a screening process for their ability and reliability to distinguish the tested film's attributes. The panelists participated in one orientation and one training session. Panelists were trained to discriminate and score consistently for the attributes being tested; turbidity, odor, sweetness, and adhesiveness. The training involved sampling several samples of varying intensities for each attributes being investigated (Appendix IV). Panelists also practiced using the structured rating scale to quantify tested attributes. Panelists were provided with feedback on their ratings. Data collection sessions were held once a day in three consecutive days. All testing and training sessions were conducted in a climate-controlled, sensory analysis laboratory equipped with individual testing booths. Panelists were provided water at room temperature (~23°C) for rinsing between samples.

Whey protein-based edible films, stored at ambient condition (23 ± 2°C, 50 ± 5% RH), were cut into 7.62 cm x 2.54 cm strips before testing. Two strips of each treatment were presented in randomized group of four. The panel was instructed to evaluate the films for turbidity, odor, sweetness, and adhesiveness. Turbidity was evaluated by

observing the sample. To determine odor, the panelists were advised to sniff the sample and allow time to rest between samples. For sweetness, the panelists were advised to taste the sample by taking the entire sample in their mouth. To determine adhesiveness, panelists were advised to place the sample between the molars and chew five times, and evaluate the forces required to remove the sample from the teeth after mastication.

Panelists evaluated each characteristic using a structured 9-point intensity scale, where 9 indicated the highest and 1 the lowest intensity of an attribute. Each attribute was rated on a separate ballot. Sensory scores were averaged for 15 judges for each treatment (for all three replicates) and attributes tested. A space for written comments was included at the bottom of the questionnaire. Sensory evaluation was conducted as approved by MSU UCRIHS for use of human subjects (Appendix IV).

5.3.7 Whey protein-based pouches for powder cocoa mix

Glycerol-plasticized WPI films without and with CW16% were selected to investigate their effectiveness as pouch packaging materials for powder cocoa mix. A pouch was manufactured from two pieces of films (cut 7.62cm x 5.08cm), and heat sealed (110°C, 40psi, 3sec) on the sides using a thermal heat sealer Model-12ASL (Sencorp system Inc., Hyannis, MA). Approximately 10 gram of powder cocoa mix was placed inside each pouch and the open end was sealed. The samples were stored at ambient condition (23 ± 2°C, 50 ± 5 % RH). Control samples included unpackaged powder cocoa mix and powder cocoa mix in its original (LDPE/foil/LDPE/paper/LDPE) package. All samples were stored and tested simultaneously.

Moisture content of the powder cocoa mix and whey protein-based film pouches were determined initially at 0 day and then every 10 days for 40 days. Powder cocoa mix was removed from the packages by cutting one end of the pouch. Approximately 3 grams of powder cocoa mix was placed in an aluminum weighing dish and weighed to obtain initial weight of samples, then dried at 100 ± 2°C for 3 h in a drying oven. After drying, samples were cooled in a desiccator for 30 min then weighed. All film samples were weighed to the nearest 0.0001gram before and after drying. Moisture contents of the whey protein-based film pouches were also determined by drying them at 100 ± 3°C for 24 h. Moisture contents of the powder cocoa mix and the whey protein-based film pouches during storage were calculated using the equation (20) in section 5.3.3 of chapter 5.

5.3.8 Statistical analysis

The moisture content, solubility in water, equilibrium moisture content, sensory attributes of the whey protein/lipid emulsion films, and the moisture content changes determination of powder cocoa mix and whey protein-based film pouches were replicated three times in a randomized block experiment. A new film forming solution and new set of films were prepared for each replicate. Statistical analysis were made using Sigma Stat 2.0 (Jandel Corp., San Rafael, CA) and appropriate comparisons were done using Student-Newman-Keuls method for multiple comparisons.

5.4 RESULTS & DISCUSSION

5.4.1 Moisture content of the films

Table 5.1 shows moisture contents of sorbitol- or glycerol-plasticized whey protein/lipid emulsion films. Overall, moisture contents of sorbitol-plasticized films were lower than those of glycerol-plasticized films, 10.1-11.4 and 13.3-16.7 %, respectively, although statistical comparisons were made only within the same plasticizer type. Non-lipid containing films had the highest moisture contents among treatments within each plasticizer type. Addition of BF4% and CW16% to films decreased ($p < 0.05$) moisture contents of sorbitol- and glycerol-plasticized WPI films (Table 5.1). However, this may be also due to the lower plasticizer content of these films since plasticizer content was subtracted from the total dry weight of films to accommodate lipids incorporation (Table 4.2).

Sorbitol and glycerol (polyhydric alcohols) are carbohydrate derivatives containing hydroxyl groups as functional groups (Appendix II). Hydroxyl groups interact with water molecules by hydrogen bonding, thus their structural differences greatly affect the rate of water bonding and the amount of water bound (Lindsay, 1985; Whistler and Daniel, 1985). Thus, it was expected that sorbitol-plasticized films would have higher moisture contents because sorbitol is a larger molecule and has more hydroxyl groups available for hydrogen bonding than glycerol. However, this was not case. The higher moisture contents of glycerol-plasticized films observed here might also be responsible for lower thermal transition temperatures (108-122°C) compared to sorbitol-plasticized films (Table 4.4) reported previously.

Table 5.1. Moisture content of whey protein/lipid emulsion edible films.

Treatments [1]	Moisture (%)
IS	11.4 ± 0.1 [a]
IS-BF4%	10.3 ± 0.4 [b]
IS-CW16%	10.1 ± 0.3 [b]
IG	16.7 ± 0.2 [a]
IG-BF4%	14.3 ± 0.5 [b]
IG-CW16%	13.3 ± 0.2 [c]

[a-c] Comparisons are made only within the same plasticizer type and different letters columnwise denotes significant difference ($p < 0.05$), $n = 3$ for all treatments.

[1] I = whey protein isolate; S = sorbitol; BF = butter fat; CW = candelilla wax; G = glycerol

5.4.2. Solubility of the films in water

Water solubilities of whey protein/lipid emulsion films are shown in Table 5.2. Statistical comparisons were made only within the films containing the same plasticizer. Sorbitol-plasticized films were dissolved after 7, 15 and 17 h depending on their compositions (Table 5.2). Time to dissolve these films in water increased ($p < 0.05$) with decreased sorbitol contents. After 24 h at 20°C, all sorbitol-plasticized films were soluble in water. On the other hand, glycerol-plasticized films were not dispersed after 24 hr immersion in water and showed no visual loss of integrity. Glycerol-plasticized films without lipids showed the highest water solubility ($p < 0.05$) while the glycerol-plasticized films with CW16% exhibited the lowest water solubility ($p < 0.05$) among the glycerol-plasticized films. These results are consistent with Cuq et al. (1997b). They reported a strong relationship between the solubilities of the myofibrillar protein-based films and their plasticizer contents: increased plasticizer contents increased solubilities of the films in water. However, addition of lipids may have also reduced films' solubility in water, because incorporation of lipids reduces hydrophilicity of polymers (McHugh and Krochta, 1994c). Less hydrophilic films are likely to interact less with water thus be less soluble. The results of this present study indicate that whey protein-based films with varying water solubilities for different applications can be obtained by monitoring plasticizer contents in film composition.

Interestingly, moisture contents (Table 5.1) of the films were not relevant to the film's solubilities in water. Glycerol-plasticized films had higher ($p < 0.05$) moisture content of 13.3-16.7 %, but showed better resistance to water than sorbitol-plasticized films, whose moisture contents ranged from 10.1 to 11.4 %.

Table 5.2. Water solubility and elapsed time to solubilize whey protein/lipid emulsion edible films (at 20° for 24hr).

Treatments [1]	Water solubility (%)	Time (hr)
IS	100 ± 0.0 [a]	7 ± 0.0 [c]
IS-BF4%	100 ± 0.0 [a]	15 ± 0.3 [b]
IS-CW16%	100 ± 0.0 [a]	17 ± 0.3 [a]
IG	31.6 ±0.7 [a]	24 ± 0.0 [a]
IG-BF4%	27.6 ± 0.5 [b]	24 ± 0.0 [a]
IG-CW16%	24.7 ± 0.3 [c]	24 ± 0.0 [a]

[a-c] Comparisons are made only within the same plasticizer type and different letters columnwise denote significant difference ($p < 0.05$), n=3 for all treatments.
[1] I = whey protein isolate; S = sorbitol; BF = butter fat; CW = candelilla wax; G = glycerol

5.4.3 Moisture sorption isotherm of the films

The moisture sorption isotherms (MSI) of sorbitol- and glycerol-plasticized films are shown in Figures 5.1 and 5.2. A slow increase in the equilibrium moisture content (EMC) of the sorbitol-plasticized WPI films from 0 to 0.7 a_w was observed followed by exponential growth peaking at around 85% RH (Figure 5.1). A similar trend was observed in glycerol-plasticized WPI films (Figure 5.2).

CW16%-added films showed the lowest EMC values in both sorbitol- and glycerol-plasticized films, while films without lipids showed the highest EMC values at all a_w levels. EMC of BF4%-added films fell between the values of the films without lipids and with CW. This indicates that incorporation of lipids (BF and CW) lowered water adsorption by WPI films, while CW being more effective than BF.

Overall, EMC values were higher for glycerol-plasticized films. This was probably due to the more hygroscopic characteristic of glycerol thus glycerol-plasticized films compared to sorbitol and sorbitol-plasticized films. This was consistent with the discussion earlier (section 5.4.1) that glycerol retained more water molecules in the films than sorbitol. The MSI of foods represents the hygroscopic properties of the components (Iglesias and Chirife, 1982). Thus, the MSI of edible films reflect hydrophilic nature of film-forming components, the plasticizer and the protein (Jangchud and Chinnan, 1999). Plasticizers are generally added during film formation to decrease intermolecular forces among polymer chains and to increase flexibility of films, however, depending on the plasticizer used this may increase hydrophilicity of the films.

The GAB model curve corresponded closely with the experimental data for the films. The GAB model parameters are shown in Table 5.3. The C and k are factors that

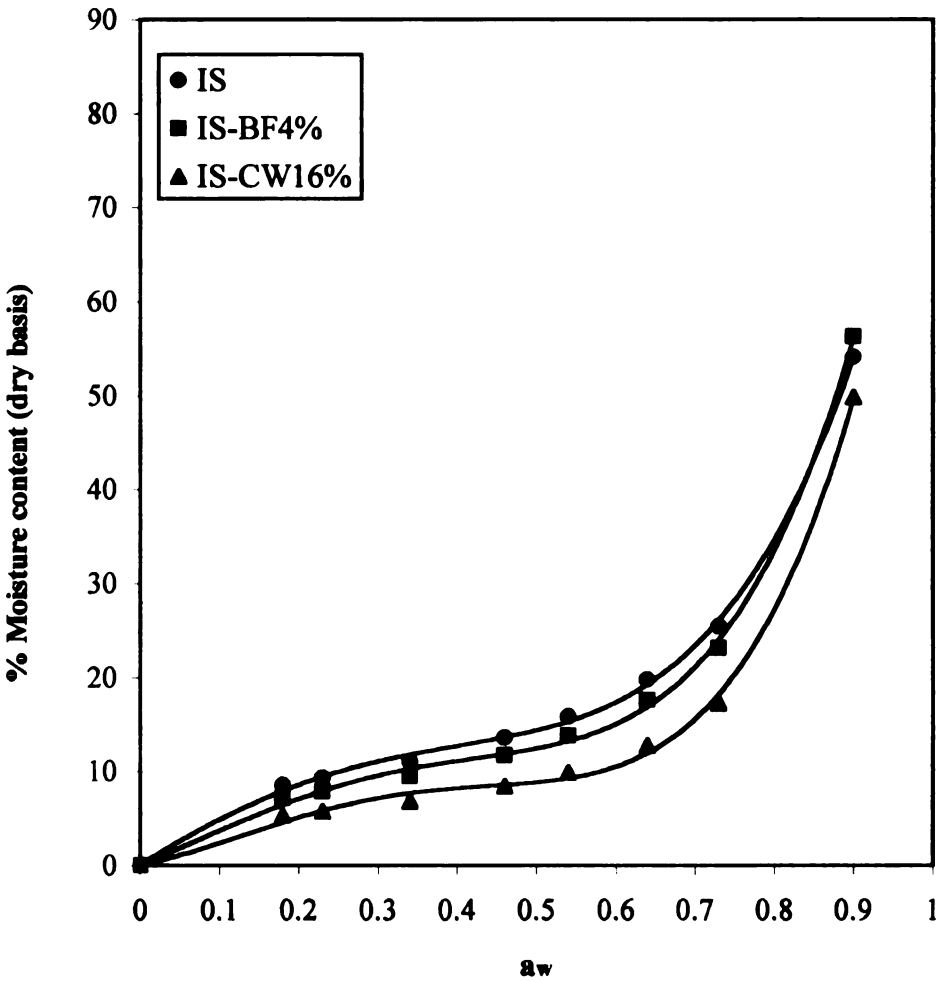


Figure 5.1. Moisture sorption isotherm of whey protein/lipid emulsion edible films plasticized with sorbitol (tested at 25°C). I = whey protein isolate; S = sorbitol; BF = butter fat; CW = candelilla wax.

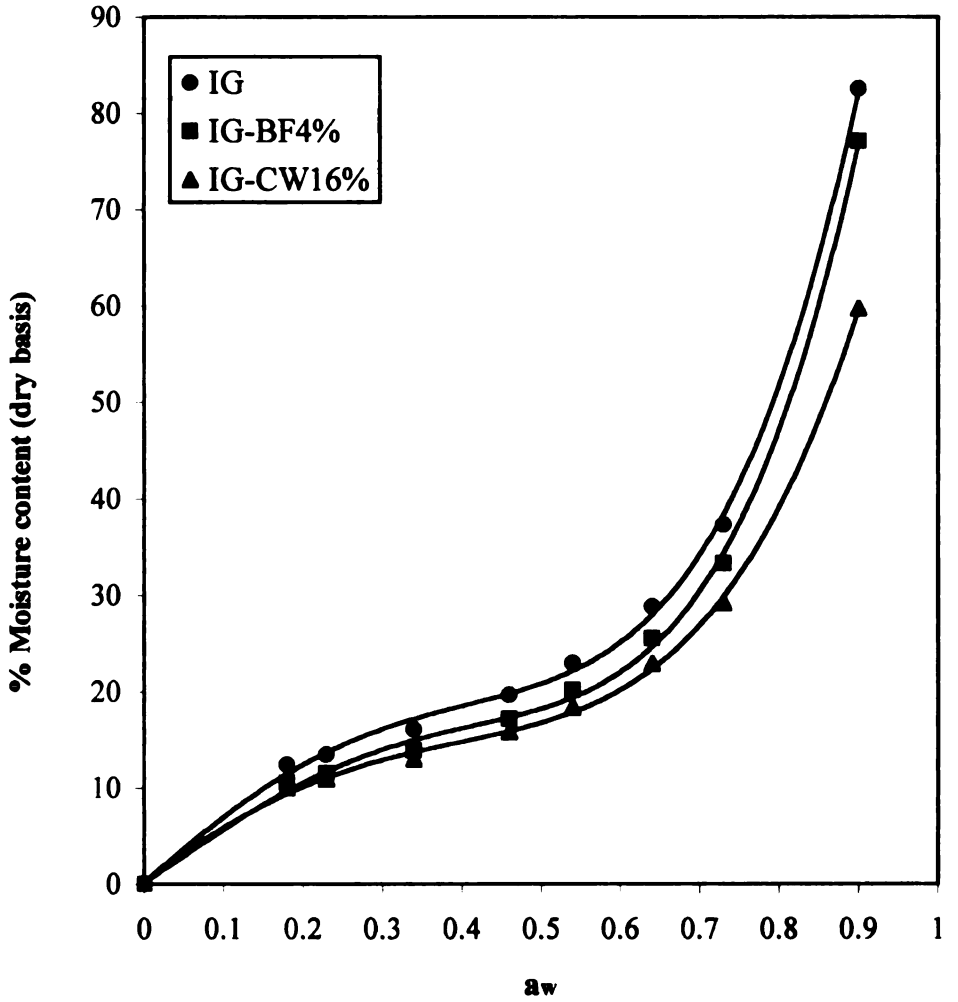


Figure 5.2. Moisture sorption isotherm of whey protein/lipid emulsion edible films plasticized with glycerol (tested at 25°C). I = whey protein isolate; G = glycerol; BF = butter fat; CW = candelilla wax.

Table 5.3. Parameters of fitted Guggenheim-Anderson-de Boer equation for whey protein/lipid emulsion edible films[1].

Treatments [2]	W_m	C	k
IS	7.88	21.92	0.95
IS-BF4%	6.77	23.83	0.98
IS-CW16%	4.54	24.01	1.01
IG	11.26	23.73	0.96
IG-BF4%	9.83	25.68	0.97
IG-CW16%	9.24	28.39	0.99

[1] W_m = monolayer moisture content;
C = a factor correcting enthalpy difference between monolayer and multilayer water;
k = a factor correcting enthalpy difference between free water and multilayer water.
[2] I = whey protein isolate; S = sorbitol; G = glycerol; CW = candelilla wax

correct for differences in enthalpy of monolayer and free water compared to that of multilayer water, respectively. W_m is the monolayer moisture content (Lim et al., 1999). Monolayer moisture is the amount of water needed to form a monolayer over the accessible polar groups of the dry matter (Fennema, 1985). The C and k parameters were increased and W_m was decreased upon decreased concentration of sorbitol or glycerol in the films. W_m values of glycerol-plasticized films were higher than those of sorbitol-plasticized films.

5.4.4 Sensory evaluation of the films

Sorbitol- and glycerol-plasticized films with BF4% felt too greasy, thus, they were left out of the sensory evaluation. Only sorbitol- and glycerol-plasticized films with and without CW16% were used for sensory evaluation by the trained sensory panels. Results of sensory evaluation: turbidity, odor, sweetness and adhesiveness, are shown in Table 5.4, and comments by the panelists are provided in Appendix IV.

In comparing turbidity (structured scale of 1-9) of the films, the trained sensory panel assigned 8.42 and 8.46 to sorbitol- and glycerol-plasticized films, respectively, with CW16%, indicating similarity of these films to wax paper (panel training scale 9) in appearance. Sorbitol- and glycerol-plasticized films without lipids received lowest scores of 2.02 and 2.07, respectively, these films possessed transparencies close to that of LDPE (panel training scale 1). Rhim et al. (1999a) also observed increased opacity of soy protein-based films upon addition of lipids (fatty acids) and reported increased whiteness of the films as determined by HunterLab Colorimeter L-value. According to Hernandez (1997), transparency or opacity of polymers is due to the morphology of the polymer, and

Table 5.4. Sensory characteristics of whey protein/lipid emulsion edible films.

Treatments [1]	Turbidity	Odor	Sweetness	Adhesiveness
IS	2.02 ± 0.79^{b}	1.38 ± 0.67^{a}	4.60 ± 1.66^{a}	3.96 ± 1.44^{a}
IS-CW16%	8.42 ± 0.57^{a}	1.64 ± 0.67^{a}	3.78 ± 1.60^{a}	3.40 ± 1.68^{a}
IG	2.07 ± 0.99^{b}	1.64 ± 1.01^{a}	5.58 ± 2.10^{a}	2.42 ± 1.19^{b}
IG-CW16%	8.64 ± 0.56^{a}	2.00 ± 1.27^{a}	3.93 ± 1.85^{a}	1.76 ± 0.80^{b}

[a-b] Comparisons are made within the same column means, with different superscripts are significantly different ($P < 0.05$), n = 45 (3 reps x 15 judges).

[1] I = whey protein isolate; S = sorbitol; G = glycerol; CW = candelilla wax

not related with their chemical structure or molecular mass. Morphological inhomogeneities of CW-added films may have caused visible light to scatter through the thus resulting in their opaqueness.

The sensory panel did not detect any specific milk odor from the films, and scored them from 1.38 to 2.0 for milk odor. These values were close to the score for purified water (panel training scale 1). These results are contrary to Morr and Ha (1993) who's reported that commercial whey protein products often have off-flavors that limit their uses. However, based on the data from the present study, I don't anticipate milk odor will be a limitation in the use of whey protein-based films as edible packaging materials.

The panel scored sweetness of the films as slightly less than 2.5% (w/v) of sugar solution (panel training scale 5), and there were no significant differences in sweetness of the films depending on the treatments. Relative sweetness of both sorbitol and glycerol is approximately 60% that of sucrose (Budavari et al., 1989), which have contributed somewhat high sweetness scores of these films.

The adhesiveness of sorbitol-plasticized films were higher than that of glycerol-plasticized films ($p < 0.05$). Addition of CW16% did not affect adhesiveness. Although it was not statistically significant, CW16%-added films were less adhesive compared to films without lipids added in both sorbitol- and glycerol-plasticized films. This may also be due to the reduction of plasticizers in these films. CW16%-added films had less plasticizer content than films without lipid added in both plasticizer types. The glycerol-plasticized films with CW16% were the least adhesive among all treatments (Table 4.2).

Overall, whey protein/lipid emulsion edible films were an acceptable in odor, sweetness, and adhesiveness characteristics as determined by a trained sensory panel.

These qualifications may extend use of whey protein-based edible films on foods as coatings, wrappings, and casings, since these indistinctive sensory characteristics will not interfere with food's taste, flavor or texture.

5.4.5 Whey protein-based pouches for powder cocoa mix

Moisture uptakes (%) of unpackaged and packaged (in commercial packages, and in glycerol-plasticized WPI pouches with and without CW16%) powder cocoa mix samples are compared in Table 5.5. Statistical comparisons were made within the same storage day of powder cocoa mix stored in different packages. Glycerol-plasticized WPI pouches with and without CW were effective in lowering ($p < 0.05$) moisture adsorption by the powder cocoa mix up to 30 days compared to that of unpackaged powder cocoa mix, however, they were not as effective as the commercial package. No differences in moisture contents were detected in powder cocoa mix packaged in the commercial package throughout the storage period of 40 days. This was expected since the commercial package was a hermetically sealed bag that can maintain integrity of the food with a long shelf life.

No significant differences were observed between the moisture contents of powder cocoa mix packaged in glycerol-plasticized WPI pouches with and without CW throughout the 40 days storage period. At 40 days of storage, moisture contents of powder cocoa mix packaged in glycerol-plasticized WPI pouches with and without CW showed no differences compared to that of unpackaged powder cocoa mix (~3%; Table 5.5). The whey protein-based pouches started cracking when stored for 40 days. These edible pouches were effective ($P < 0.05$), however, up to 30 days of storage indicating

Table 5.5. Moisture content of powder cocoa mix packaged in whey protein-based pouches stored for 40 days (23°C, 50% RH).

Treatment[1]	Moisture (%)				
	0 day	10 days	20 days	30 days	40 days
Unpackaged	2.03 ± 0.02 [a]	2.72 ± 0.06 [a]	2.92 ± 0.08 [a]	2.96 ± 0.04 [a]	2.95 ± 0.08 [a]
Commercial package	2.03 ± 0.02 [a]	2.03 ± 0.01 [c]	2.03 ± 0.02 [c]	2.03 ± 0.02 [c]	2.03 ± 0.01 [c]
packaged in IG film pouch	2.03 ± 0.02 [a]	2.37 ± 0.07 [b]	2.51 ± 0.02 [b]	2.67 ± 0.07 [b]	2.91 ± 0.08 [a]
packaged in IG-CW film pouch	2.03 ± 0.02 [a]	2.36 ± 0.13 [b]	2.54 ± 0.13 [b]	2.74 ± 0.05 [b]	3.01 ± 0.07 [a]

[a-c] Comparisons are made within the same column means with different superscripts are significantly different ($P < 0.05$), n = 3 for all treatments.
[1] I = whey protein isolate; G = glycerol; CW = candelilla wax.

Table 5.6. Moisture content of whey protein-based pouches used to store powder cocoa mix for 40 days (23°C, 50% RH).

Treatment[1]	Moisture (%)				
	0 day	10 days	20 days	30 days	40 days
IG film pouch	16.7 ± 0.2 [a]	11.5 ± 1.3 [b]	11.0 ± 0.7 [b]	10.6 ± 0.8 [b]	10.3 ± 1.4 [b]
IG-CW film pouch	13.3 ± 0.2 [a]	12.2 ± 1.4 [a]	10.7 ± 0.7 [b]	9.9 ± 0.3 [b]	10.1 ± 0.3 [b]

[a-b] Comparisons are made within the same raw means with different superscripts are significantly different ($P < 0.05$), n = 3 for all treatments.

[1] I = whey protein isolate; G = glycerol; CW = candelilla wax.

that whey protein-based films could provide protection to a food product like powder cocoa mix after the primary packaging is opened until this time.

Moisture content changes of pouches used to store powder cocoa mix for 40 days are shown in Table 5.6. The moisture content of glycerol-plasticized WPI pouches decreased significantly ($p < 0.05$) after 10 days (from 16.7 to 11.5%) and then remained the same throughout the storage period. A similar trend was observed for glycerol-plasticized WPI pouches with CW, however this decrease was observed after 20 days instead. Cracking of these pouches probably occurred due to moisture loss from the pouch materials, thus made them brittle and crack. Moisture contents of both pouches were ~10% after 40 days storage period. This value was lower than what was expected at the 50% RH storage condition.

Powder cocoa mix probably absorbed moisture from the WPI films as well as from the environment. Powder cocoa mix used in this present study contains approximately 68% sugar (w/w of serving size; value provided by Nestlé Inc.), which makes it fairly hygroscopic (Whistler and Daniel, 1985). Better results probably would have been obtained if these films were used for packaging of non-hygroscopic foods such as pasta, oatmeal, mashed potatoes flake, etc. (Figure 5.3). Their stability when packaged in whey protein-based pouches have not yet been determined.

Studies were also conducted to investigate the possible use of whey protein-based films as a wrap for processed cheese slices. These experiments and results are presented in Appendix V. Overall, whey protein-based films were ineffective as wraps for processed cheese slices.

Figure 5.3. Various food products in pouches manufactured using whey protein-based edible films.

In summary, moisture contents of the whey protein-based films were mainly influenced by plasticizer type, but within the same plasticizer type, concentration of the plasticizer affected the moisture contents of the films. Solubilities of the films were also influenced primarily by plasticizer type and contents. Although no statistical comparisons were made, glycerol-plasticized films appeared to be less soluble than sorbitol-plasticized films. Incorporation of lipids effectively lowered water adsorption of the films and formed less hygroscopic films as determined by the MSI. Whey protein-based films were slightly sweet and adhesive but lacking in milk odor. Incorporation of CW resulted in opaque films but did not affect odor, taste or adhesiveness of the films as determined by a trained sensory panel. Whey protein/lipid emulsion edible films were suitable for packaging of powder cocoa mix up to 30 days and may be more suitable for packaging of non-hygroscopic foods.

CONCLUSIONS

1. Whey protein/lipid emulsion films containing butter fat (BF) and candelilla wax (CW) showed better water vapor permeability (WVP) and oxygen permeability (OP) than non-lipid containing films.

2. Lipid concentration affected WVP of the films. Overall, WVP decreased with increase in lipid concentration. Most desirable WVP was observed in WPI films containing CW16%.

3. OP of the films were significantly influenced by lipid type, rather than lipid concentration or whey protein type.

4. Mechanical properties of whey protein/lipid emulsion films were influenced by protein and lipid types as well as lipid concentration. Overall, whey protein isolate (WPI) films with CW showed better tensile strength than whey protein concentrate (WPC) films with BF.

5. Scanning Electron Micrographs elucidated relationship between microstructure, and barrier and mechanical properties of the films.

6. The main forces involved in the formation and stability of whey protein and whey protein/lipid emulsion films were disulfide and hydrogen bonds. Contribution of hydrophobic interactions to their formation and stability was minimal.

7. Protein types affected free sulfhydryl group contents of the films: 2.5 to 3.0 μmol/g of film for WPI films, and 1.39 to 1.44 μmol/g of film for WPC films. Disulfide bond contents, although not statistically different were lower in WPC films than in WPI films. Lower disulfide contents of WPC films coincided with lower TS values of the

films, while higher disulfide contents of WPI films agreed with higher TS values of the films.

8. Hydrophobicity of the films increased with incorporation of lipids. Although CW containing films were more hydrophobic than BF containing films, these differences were not statistically significant. Hydrophobicity of the films coincided with reduced WVP of the films upon addition of lipids. Films that showed the lowest WVP had the highest hydrophobicity values.

9. Differential Scanning Calorimetry showed onset transition temperature (T_o) of 108-122°C for the glycerol-plasticized WPI films, and 126-127°C for the sorbitol-plasticized WPI films.

10. All films were heat sealable. The seal strengths of the films ranged from 110 to 323 N/m. Pressure and dwell time variation did not affect seal strength of the films. However, the plasticizer type influenced optimum heat-sealing temperature of the films, 130°C for sorbitol-plasticized and 110°C for glycerol-plasticized WPI films.

11. Electron Spectroscopy for Chemical Analysis (ESCA) was used to study the nature of the interfacial interactions in heat sealed and unsealed whey protein/lipid emulsion edible films. Main interfacial interactions on the surfaces of heat sealed whey protein-based films appeared to be hydrogen and covalent bonds involving C-O-H and N-C components, respectively.

12. Moisture contents of the whey protein/lipid emulsion films were mainly influenced by plasticizer type, but within the same plasticizer type, concentration of plasticizer affected moisture contents of the films. Overall, glycerol-plasticized films showed higher moisture content compared to those of sorbitol-plasticized films.

13. Solubilities of the films in water were primarily influenced by the plasticizer content. The higher the plasticizer content, the greater was the solubility of the films in water.

14. Incorporation of lipids effectively lowered water adsorption of the films and formed less hygroscopic films as determined by moisture sorption isotherm.

15. Sensory evaluation of the films revealed that no distinctive milk odor existed in whey protein and whey protein/lipid emulsion films. However, the films were perceived to be slightly sweet and adhesive by the panelists. Addition of CW resulted in opaque films.

16. Whey protein/lipid emulsion edible films may be suitable for packaging of powder cocoa mix and should be suitable for packaging of non-hygroscopic foods.

APPENDIX I

Appendix I. Amino acid composition of β-lactoglobulin and α-lactoalbumin (Swaisgood, 1985).

Amino acid	**β-Lactoglobulin A**	**α-Lactoalbumin B**
Alanine (Ala, A)	14	3
Isoleucine (Ile, I)*	10	8
Leucine (Leu, L)*	22	13
Methionine (Met, M)*	4	1
Phenylalanine (Phe, F)*	4	4
Proline (Pro, P)	8	2
Tryptophan (Trp, W)*	2	4
Valine (Val, V)*	10	6
Asparagine (Asn, N)	5	12
Cysteine (Cys, C)	5	8
Glutamine (Gln, Q)	9	5
Glycine (Gly, G)	3	6
Histidine (His, H)*	2	3
Serine (Ser, S)	7	7
Threonine (Thr, T)*	8	7
Tyrosine (Tyr, Y)	4	4
Aspartic acid (Asp, D)	11	9
Glutamic acid (Glu, E)	16	8
Arginine (Arg, R)*	3	1
Lysine (Lys, K)*	15	12
Total residues	**162**	**123**

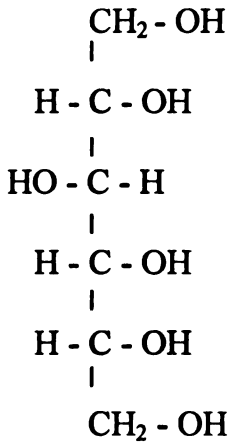
* Essential amino acids

APPENDIX II

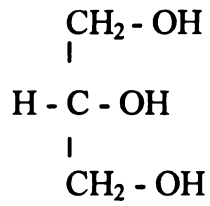
Appendix II. Structures of polyol plasticizers.

A. Sorbitol (hexahydric alcohol); **B.** Glycerol (trihydric alcohol).

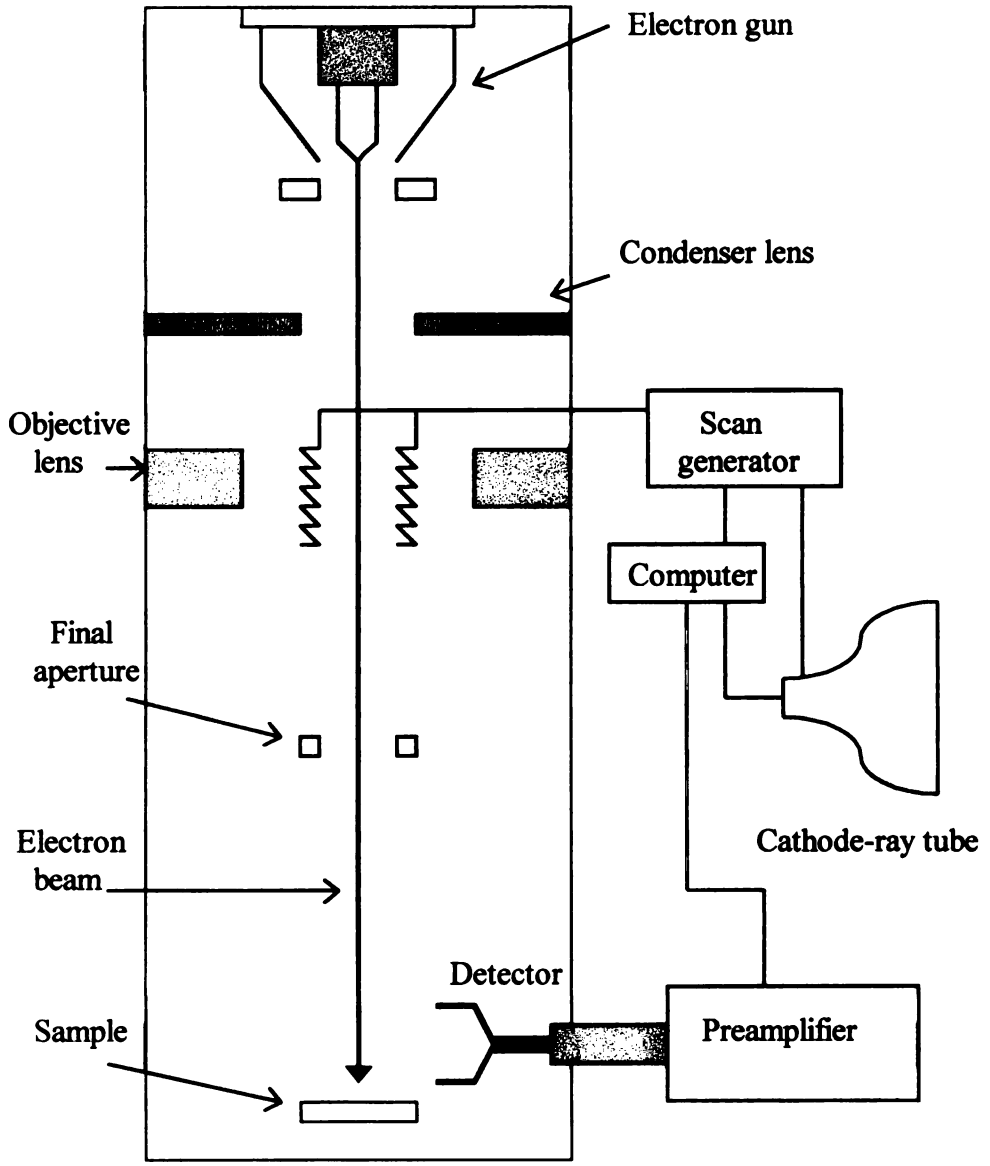
A. Sorbitol



B. Glycerol



APPENDIX III



Appendix III. Schematic diagram of scanning electron microscope (SEM) (Flegler et al., 1993).

APPENDIX IV

Written consent form

Department of Food Science and Human Nutrition
Michigan State University

Edible films prepared from whey protein isolate, sorbitol, glycerol, candelilla wax and water.

I _____ have read the above list of ingredients and find none that I am allergic to. I have also been informed on the nature of the research (including experimental materials and procedures) which will be used during the tasting session. I understand that the taste panel will take approximately 10-15 minutes. I agree to serve on the taste panel, which will be conducted on _____, 1999. I understand that I am free to withdraw my consent and to discontinue participation in the panel at any time without penalty.

UCRIHS APPROVAL FOR THIS project EXPIRES:

FEB 17 2000

SUBMIT RENEWAL APPLICATION ONE MONTH PRIOR TO ABOVE DATE TO CONTINUE

_____
Signature

_____
Date

Advertisement

Need
Whey protein-based edible films
Sensory panel

Monday, Feb. 22, 1999
1:00 p.m. - 2:00 p.m.

and

Monday, March 1, 1999
1:00 p.m. - 2:00 p.m.

Take 5-10 minutes out of your day
to try a new product and earn a treat for helping us out.
Just stop by at any time during the above listed times.

Trained panel prescreening questionnaire

Name _____

Phone (Day) _____ (Evening) _____

Gender M ____ or F_____

Age 18-25 ___, 26-35 ___, 36-55 ___, >55 ___

Time

1. Do you plan to be on campus during this semester? ___
2. Are there any weekdays that you will not be available on a regular basis?
3. What part of the day are you normally available?
   Morning (8-11) ___
   Early afternoon (11-2) ___
   Afternoon (2-5) ___

Health

1. Do you have any food allergies (specifically to milk proteins) ?
2. Do you take any medications which affect your senses?
3. Are you currently on a restricted diet? If yes, please explain.
4. What foods (specifically dairy foods) can you not eat?
5. What foods (specifically dairy foods) do you not like to eat?

Thank you for your time!

Questionnaire for panel training

Name : _____ Date : _____

Characteristics studied : **Turbidity**

Please evaluate two samples by observing turbidity. Place an X next to the value which best describe the turbidity of the samples.

Sample : _____

Turbidity

___9 Opaque
___8
___7
___6
___5 Moderate
___4
___3
___2
___1 Transparent

Sample : _____

Turbidity

___9 Opaque
___8
___7
___6
___5 Moderate
___4
___3
___2
___1 Transparent

Questionnaire for panel training

Name : _____ Date : _____

Characteristics studied : **Odor**

Please evaluate the two samples by sniffing. Allow time to rest between samples. Place an X next to the value which best describe the odor of the samples.

Sample : _____

Odor

___9 Strong
___8
___7
___6
___5 Moderate
___4
___3
___2
___1 Weak

Sample : _____

Odor

___9 Strong
___8
___7
___6
___5 Moderate
___4
___3
___2
___1 Weak

Questionnaire for panel training

Name : _____ Date : _____

Characteristics studied : **Sweetness**

Please rinse your mouth with water before starting. There are two samples for you to evaluate. Taste each of the coded samples by taking the entire sample in your mouth. Rinse your mouth with water between samples and expectorate all samples and water. Place an X next to the value which best describe the sweetness of the samples.

Sample : _____

Sweetness

___9 Sweet
___8
___7
___6
___5 Moderate
___4
___3
___2
___1 Not sweet

Sample : _____

Sweetness

___9 Sweet
___8
___7
___6
___5 Moderate
___4
___3
___2
___1 Not sweet

Questionnaire for panel training

Name : _____ Date : _____

Characteristics studied : **Adhesiveness**

Please rinse your mouth with water before starting. There are two samples for you to evaluate. Place sample between molars and chew five times. Evaluate the force required to remove the sample from the teeth after mastication of the product. Rinse your mouth with water between samples and expectorate all samples and water. Place an X next to the value which best describe the adhesiveness of the samples.

Sample : _____

Adhesiveness

___9 Very adhesive
___8
___7
___6
___5 Moderate
___4
___3
___2
___1 Not adhesive

Sample : _____

Adhesiveness

___9 Very adhesive
___8
___7
___6
___5 Moderate
___4
___3
___2
___1 Not adhesive

Trained panel questionnaire

Product: Whey protein-based edible film
Name : _____ Date : _____

1. Please evaluate the whey protein-based edible films by observing and tasting each sample in the following order. Taste the sample and rinse with water between tasting. Place an X next to the value which best describes the characteristic intensity of the sample.

Sample number : ____

Turbidity	**Odor**	**Sweetness**	**Adhesiveness**
___9 Opaque	___9 Strong	___9 Sweet	___9 Very adhesive
___8	___8	___8	___8
___7	___7	___7	___7
___6	___6	___6	___6
___5 Moderate	___5 Moderate	___5 Moderate	___5 Moderate
___4	___4	___4	___4
___3	___3	___3	___3
___2	___2	___2	___2
___1 Transparent	___1 Weak	___1 Not sweet	___1 Not adhesive

Comments : _____

Sample number : ____

Turbidity	**Odor**	**Sweetness**	**Adhesiveness**
___9 Opaque	___9 Strong	___9 Sweet	___9 Very adhesive
___8	___8	___8	___8
___7	___7	___7	___7
___6	___6	___6	___6
___5 Moderate	___5 Moderate	___5 Moderate	___5 Moderate
___4	___4	___4	___4
___3	___3	___3	___3
___2	___2	___2	___2
___1 Transparent	___1 Weak	___1 Not sweet	___1 Not adhesive

Comments : _____

Sample number : ____

Turbidity	**Odor**	**Sweetness**	**Adhesiveness**
___9 Opaque	___9 Strong	___9 Sweet	___9 Very adhesive
___8	___8	___8	___8
___7	___7	___7	___7
___6	___6	___6	___6
___5 Moderate	___5 Moderate	___5 Moderate	___5 Moderate
___4	___4	___4	___4
___3	___3	___3	___3
___2	___2	___2	___2
___1 Transparent	___1 Weak	___1 Not sweet	___1 Not adhesive

Comments : _____

Sample number : ____

Turbidity	**Odor**	**Sweetness**	**Adhesiveness**
___9 Opaque	___9 Strong	___9 Sweet	___9 Very adhesive
___8	___8	___8	___8
___7	___7	___7	___7
___6	___6	___6	___6
___5 Moderate	___5 Moderate	___5 Moderate	___5 Moderate
___4	___4	___4	___4
___3	___3	___3	___3
___2	___2	___2	___2
___1 Transparent	___1 Weak	___1 Not sweet	___1 Not adhesive

Comments : _____

Panel training samples codes

Attributes	Hedonic scale	Samples	Codes
Turbidity	9	Wax paper	285
	3	IS film	516
	1	LDPE	949
Odor	9	8% WPI soln.	491
	5	4% WPI soln.	149
	1	Purified water	981
Sweetness	9	5% Sugar soln.	620
	5	2.5% Sugar soln.	352
	1	Purified water	778
Adhesiveness	9	Caramel	257
	6	Jelly bean	185
	1	Gummi-bear	564

Trained panel treatments codes

Treatment	Rep #1 code	Rep #2 code	Rep #3 code
IS	435	585	885
IS-CW16	122	151	117
IG	644	974	394
IG-CW16	893	628	931

Trained panel comments

IS film

Rep #1 - Slightly sweet when first tasted

Rep #2 - Stronger whey taste.

- Sweet taste initially

- Milky taste

Rep #3 - More adhesive than other samples

- Dissolve relatively easily

IG film

Rep #1 - Slightly sweet at first, not really adhesive

Rep #2 - Sweet taste initially

- Milky taste, breaks upon chewing

Rep #3 - Sweetest sample tasted.

IS-CW16 film

Rep #1 - A little bitterness tasted after chewing

- Slightly sweet after a while

- It has some milky flavor.

Rep #2 - Bad after taste.

Rep #3 - Sweet taste later on.

IG-CW16 film

Rep #1 - Doesn't dissolve well in the mouth

- No taste, not really adhesive

- After chewing, it disintegrate, different texture

Rep #2 - Disintegrate easily

APPENDIX V

WHEY PROTEIN-BASED EDIBLE FILMS AS A CHEESE WRAP FOR PROCESSED CHEESE SLICES

Materials

Whey protein isolate (WPI; ALACEN 895) was provided by New Zealand Milk Products (North America) Inc. (Santa Rosa, CA). Candelilla wax (CW) was purchased form Strahl and Pitch Inc. (West Babylon, NY). D-Sorbitol was obtained from Sigma Chemical Co. (St. Louis, MO), and NaOH was purchased from Mallinkrodt Specialty Chemical Co. (Paris, KY). Kraft singles® American processed cheese slices (Kraft Foods Inc., Glenview, IL), and Ziploc® freezer bags (Dow Chemical Co., Indianapolis, IN) were purchased at a local retail outlet (E. Lansing, MI).

Methods

Film preparation The sorbitol-plasticized WPI films and CW16%-added films, showed the best moisture barrier property (Table 2.3 in chapter 2), thus were selected as cheese wraps to investigate their effectiveness on moisture loss from processed cheese slices. Both whey protein films were produced as described in Figure 2.1 in chapter 2., and composition of the film produced were shown in Table 2.2 of chapter 2.

Preparation of cheese wraps Cheese slices (8.26cm x 7.87cm) were placed between two layers of sorbitol-plasticized WPI films and CW16%-added films, then the edge of the films were sealed using a thermal heat sealer Model-12ASL (Sencorp System Inc., Hyannis, MA) at 130°C, 60psi for 3sec. This heat sealing condition was selected based on the results from the seal strength study (Table 3.5 in chapter 3). Cheese wrapped with sorbitol-plasticized WPI films and CW16%-added films were placed

individually inside Ziploc® polyethylene bags (17.78 cm x 20.32 cm) and stored in the chamber with ambient conditions. Effectiveness of whey protein films as cheese wraps were determined at 4.0 ± 1.0°C in two different RH conditions of 10 ± 3 and 88 ± 5%. Another set of cheeses wrapped in whey protein films but without being packed in polyethylene bags were stored in both RH. Control samples included unwrapped cheeses and cheeses in their original package, LDPE, were stored and tested simultaneously. The samples stored in 10% RH condition was tested for moisture loss and color change every 3 days for 9 days. The samples in 88% RH storage condition were tested every 5 days for 15 days.

Moisture content "*Standard Methods for the Examination of Dairy Products*" were used to determine the moisture content of the cheese slices (Marshall, 1992). Shredded 3.0 ± 0.5 g of cheese was placed in an aluminum weighing dish and dried for 16 hrs at 80 ± 3°C using gravity convention oven (Precision Instruments, Chicago, IL), until a constant weight was reached. After the drying, samples were placed in a dessicator for 30 min to equilibrate to room temperature and weighed. Moisture content of the whey protein films were determined by drying 3.0 ± 0.5 g of the films for 24 hrs at 100 ± 3°C. Moisture content (% Moisture) was calculated using the equation (12) in chapter 5.

Color test HunterLab colorimeter (Hunter Associates Laboratory, Inc., Reston, VA) were used to test color change of the cheeses. A black and a white standard tile were used for calibration, and the black tile as the background when testing samples. Values of L (black to white), a (green to red), and b (blue to yellow) were determined.

Statistical analysis

Experiments for moisture loss of cheeses, color change, and moisture contents of films were replicated three times in a randomized block experiment. A new film forming solution and new set of films were prepare for each replicate. Statistical analysis were made using Sigma Stat 2.0 (Jandel Corp., San Rafael, CA) and the appropriate comparisons were done using the Student-Newman-Keuls method for multiple comparisons.

Table V.1. Effect of wrap type on moisture content of processed cheese slices packaged in various wraps (4°C).

a)

Wrap types [1]	Moisture (%) at 10% RH			
	0 day	3 days	6 days	9 days
Unwrapped	40.3 ± 0.3 [a]	16.2 ± 1.1 [c]	10.7 ± 0.4 [d]	10.3 ± 0.3 [d]
Commercial package	40.3 ± 0.3 [a]	39.9 ± 0.6 [a]	39.6 ± 0.4 [a]	39.1 ± 0.9 [a]
IS film wrap	40.3 ± 0.3 [a]	16.0 ± 0.1 [c]	10.8 ± 0.1 [d]	10.3 ± 0.3 [d]
IS-CW film wrap	40.3 ± 0.3 [a]	13.2 ± 0.3 [d]	10.2 ± 0.3 [d]	10.8 ± 0.5 [d]
IS film wrap/ PE	40.3 ± 0.3 [a]	30.9 ± 0.6 [b]	31.5 ± 0.3 [b]	29.6 ± 0.3 [c]
IS-CW film wrap/ PE	40.3 ± 0.3 [a]	31.5 ± 0.2 [b]	30.4 ± 0.4 [c]	30.4 ± 0.7 [b]

b)

Wrap types [1]	Moisture (%) at 88% RH			
	0 day	5 days	10 days	15 days
Unwrapped	40.3 ± 0.3 [a]	35.9 ± 0.7 [b]	34.3 ± 0.6 [b]	32.7 ± 0.8 [b]
Commercial package	40.3 ± 0.3 [a]	38.8 ± 0.5 [a]	39.2 ± 0.5 [a]	39.3 ± 1.0 [a]
IS film wrap	40.3 ± 0.3 [a]	32.4 ± 0.5 [c]	33.1 ± 0.3 [c]	31.7 ± 0.7 [b]
IS-CW film wrap	40.3 ± 0.3 [a]	31.0 ± 0.1 [d]	30.1 ± 0.4 [d]	33.0 ± 0.7 [b]
IS film wrap/ PE	40.3 ± 0.3 [a]	29.7 ± 0.8 [e]	29.0 ± 0.5 [e]	30.2 ± 0.8 [c]
IS-CW film wrap/ PE	40.3 ± 0.3 [a]	29.1 ± 0.7 [e]	29.6 ± 1.0 [de]	31.1 ± 0.9 [bc]

[a-e] Comparisons are made within the same column means with different superscripts are significantly different ($P < 0.05$), $n = 3$ for all treatments.

[1] I = whey protein isolate; S = sorbitol; CW = candelilla wax; PE = polyethylene.

Table V.2. Effect of storage time on moisture content of edible films used as processed cheese slice wraps (4°C).

a)

Wrap types [1]	Moisture (%) at 10% RH			
	0 day	3 days	6 days	9 days
IS film wrap	11.4 ± 0.1^{d}	21.7 ± 0.8^{a}	15.9 ± 0.1^{b}	13.2 ± 0.1^{c}
IS-CW film wrap	10.1 ± 0.3^{c}	19.1 ± 0.2^{a}	15.5 ± 0.1^{b}	13.7 ± 1.8^{b}
IS film wrap/ PE	11.4 ± 0.1^{b}	42.7 ± 0.2^{a}	42.9 ± 0.5^{a}	41.9 ± 0.4^{a}
IS-CW film wrap/ PE	10.1 ± 0.3^{c}	42.7 ± 0.6^{a}	42.0 ± 0.4^{a}	39.0 ± 0.6^{b}

b)

Wrap types [1]	Moisture (%) at 88% RH			
	0 day	5 days	10 days	15 days
IS film wrap	11.4 ± 0.1^{d}	47.2 ± 0.6^{b}	48.0 ± 0.1^{a}	44.3 ± 0.3^{c}
IS-CW film wrap	10.1 ± 0.3^{c}	41.3 ± 0.2^{a}	40.5 ± 0.3^{b}	40.6 ± 0.2^{b}
IS film wrap/ PE	11.4 ± 0.1^{c}	40.0 ± 0.7^{b}	42.4 ± 0.6^{a}	43.6 ± 1.2^{a}
IS-CW film wrap/ PE	10.1 ± 0.3^{c}	39.5 ± 0.3^{b}	39.8 ± 0.5^{b}	40.5 ± 0.3^{a}

[a-d] Comparisons are made within the same raw means with different superscripts are significantly different ($P < 0.05$), $n = 3$ for all treatments.

[1] I = whey protein isolate; S = sorbitol; CW = candelilla wax; PE = polyethylene.

Table V.3. Effect of wrap type on color changes of processed cheese slices during storage (4°C, 10% RH).

a) L-value (0 black to 100 white)

Wrap types [1]	0 day	3 days	6 days	9 days
Unwrapped	64.7 ± 0.6 [a]	52.5 ± 1.0 [c]	56.0 ± 1.3 [c]	56.9 ± 0.8 [c]
Commercial package	64.7 ± 0.6 [a]	65.2 ± 0.7 [a]	64.1 ± 0.4 [a]	64.9 ± 0.5 [a]
IS film wrap/ PE	64.7 ± 0.6 [a]	60.9 ± 0.6 [b]	60.7 ± 0.7 [b]	59.2 ± 0.7 [b]
IS-CW film wrap/ PE	64.7 ± 0.6 [a]	60.6 ± 0.7 [b]	60.7 ± 0.4 [b]	60.2 ± 0.3 [b]

b) a-value (- green to + red)

Wrap types [1]	0 day	3 days	6 days	9 days
Unwrapped	5.43 ± 0.41 [a]	9.63 ± 0.34 [a]	9.56 ± 0.81 [a]	9.60 ± 0.54 [a]
Commercial package	5.43 ± 0.41 [a]	5.68 ± 0.41 [c]	5.23 ± 0.36 [c]	5.37 ± 0.15 [c]
IS film wrap/ PE	5.43 ± 0.41 [a]	7.30 ± 0.32 [b]	6.65 ± 0.41 [b]	6.53 ± 0.37 [b]
IS-CW film wrap/ PE	5.43 ± 0.41 [a]	7.00 ± 0.41 [b]	6.51 ± 0.31 [b]	6.38 ± 0.31 [b]

c) b-value (- blue to + yellow)

Wrap types [1]	0 day	3 days	6 days	9 days
Unwrapped	30.1 ± 0.2 [a]	26.4 ± 0.6 [c]	27.4 ± 0.5 [c]	27.5 ± 0.6 [c]
Commercial package	30.1 ± 0.2 [a]	29.0 ± 0.4 [a]	29.3 ± 0.4 [a]	29.4 ± 0.3 [a]
IS film wrap/ PE	30.1 ± 0.2 [a]	28.1 ± 0.6 [b]	28.6 ± 0.4 [b]	28.3 ± 0.4 [b]
IS-CW film wrap/ PE	30.1 ± 0.2 [a]	27.9 ± 0.4 [b]	28.5 ± 0.2 [b]	28.3 ± 0.3 [b]

[a-c] Comparisons are made within the same column means, with different superscripts are significantly different ($P < 0.05$), $n = 3$ for all treatments.

[1] I = whey protein isolate; S = sorbitol; CW = candelilla wax; PE = polyethylene.

Table V.4. Effect of wrap type on color changes of processed cheese slices during storage (4°C, 88% RH).

a) L-value (0 black to 100 white)

Wrap types [1]	0 day	5 days	10 days	15 days
Unwrapped	64.7 ± 0.6 [a]	63.0 ± 1.3 [b]	61.4 ± 1.0 [b]	59.7 ± 0.6 [b]
Commercial package	64.7 ± 0.6 [a]	64.3 ± 0.9 [a]	65.0 ± 0.7 [a]	65.4 ± 0.8 [a]
IS film wrap	64.7 ± 0.6 [a]	59.7 ± 0.7 [c]	60.3 ± 0.4 [c]	58.8 ± 0.5 [b]
IS-CW film wrap	64.7 ± 0.6 [a]	58.8 ± 0.5 [c]	56.3 ± 0.4 [d]	56.6 ± 0.8 [c]
IS film wrap/ PE	64.7 ± 0.6 [a]	57.2 ± 0.5 [d]	56.7 ± 0.3 [d]	57.3 ± 0.6 [c]
IS-CW film wrap/ PE	64.7 ± 0.6 [a]	57.4 ± 0.5 [d]	57.1 ± 0.7 [d]	56.8 ± 0.3 [c]

b) a-value (- green to + red)

Wrap types [1]	0 day	5 days	10 days	15 days
Unwrapped	5.43 ± 0.41 [a]	6.34 ± 0.69 [ab]	5.57 ± 0.72 [ab]	5.28 ± 0.49 [a]
Commercial package	5.43 ± 0.41 [a]	5.97 ± 0.51 [ab]	4.05 ± 0.57 [c]	3.93 ± 0.35 [b]
IS film wrap	5.43 ± 0.41 [a]	6.52 ± 0.26 [a]	4.93 ± 0.31 [b]	5.56 ± 0.51 [a]
IS-CW film wrap	5.43 ± 0.41 [a]	5.80 ± 0.37 [b]	5.87 ± 0.19 [a]	5.68 ± 0.57 [a]
IS film wrap/ PE	5.43 ± 0.41 [a]	6.25 ± 0.35 [ab]	4.97 ± 0.16 [b]	5.42 ± 0.40 [a]
IS-CW film wrap/ PE	5.43 ± 0.41 [a]	5.62 ± 0.23 [b]	5.30 ± 0.18 [ab]	5.20 ± 0.82 [a]

c) b-value (- blue to + yellow)

Wrap types [1]	0 day	5 days	10 days	15 days
Unwrapped	30.1 ± 0.2 [a]	30.7 ± 0.4 [a]	31.2 ± 0.5 [a]	31.6 ± 0.7 [a]
Commercial package	30.1 ± 0.2 [a]	30.0 ± 0.6 [b]	31.4 ± 0.4 [a]	31.5 ± 0.6 [a]
IS film wrap	30.1 ± 0.2 [a]	30.6 ± 0.6 [a]	31.0 ± 0.4 [a]	30.7 ± 0.5 [b]
IS-CW film wrap	30.1 ± 0.2 [a]	29.8 ± 0.3 [b]	29.7 ± 0.4 [b]	30.8 ± 0.2 [ab]
IS film wrap/ PE	30.1 ± 0.2 [a]	29.9 ± 0.4 [b]	29.8 ± 0.3 [b]	30.3 ± 0.6 [b]
IS-CW film wrap/ PE	30.1 ± 0.2 [a]	29.6 ± 0.3 [b]	29.9 ± 0.5 [b]	30.1 ± 0.3 [b]

[a-d] Comparisons are made within the same column means, with different superscripts are significantly different ($P < 0.05$), $n = 3$ for all treatments.

[1] I = whey protein isolate; S = sorbitol; CW = candelilla wax; PE = polyethylene.

Table V.5. Effect of storage time on color changes of whey protein films as processed cheese slice wraps (4°C, 88% RH).

a) L-value (0 transparent to 100 translucent)

Wrap types [1]	0 day	5 days	10 days	15 days
IS film wrap	6.83 ± 0.25 [b]	18.2 ± 1.6 [a]	19.1 ± 0.5 [a]	19.3 ± 0.8 [a]
IS-CW film wrap	29.3 ± 1.3 [b]	42.5 ± 1.1 [a]	42.8 ± 1.3 [a]	43.9 ± 1.1 [a]
IS film wrap/ PE	6.83 ± 0.25 [b]	19.7 ± 1.7 [a]	20.9 ± 0.8 [a]	20.1 ± 1.1 [a]
IS-CW film wrap/ PE	29.3 ± 1.3 [b]	42.4 ± 1.0 [a]	43.2 ± 0.6 [a]	43.3 ± 1.2 [a]

b) a-value (- green to + red)

Wrap types [1]	0 day	5 days	10 days	15 days
IS film wrap	- 0.90 ± 0.43 [a]	- 0.83 ± 0.24 [a]	- 1.60 ± 0.20 [b]	- 1.88 ± 0.31 [b]
IS-CW film wrap	- 1.47 ± 0.50 [a]	- 3.32 ± 0.22 [b]	- 4.10 ± 0.67 [c]	- 4.34 ± 0.17 [c]
IS film wrap/ PE	- 0.90 ± 0.43 [a]	- 1.28 ± 0.15 [a]	- 2.25 ± 0.27 [b]	- 2.08 ± 0.57 [b]
IS-CW film wrap/ PE	- 1.47 ± 0.50 [a]	- 3.67 ± 0.24 [b]	- 4.46 ± 0.11 [c]	- 4.57 ± 0.19 [c]

c) b-value (- blue to + yellow)

Wrap types [1]	0 day	5 days	10 days	15 days
IS film wrap	0.57 ± 0.29 [b]	0.65 ± 0.44 [b]	1.52 ± 0.22 [a]	1.92 ± 0.26 [a]
IS-CW film wrap	0.53 ± 0.55 [c]	3.89 ± 0.29 [b]	4.37 ± 0.30 [a]	5.38 ± 0.62 [a]
IS film wrap/ PE	0.57 ± 0.29 [c]	1.52 ± 0.28 [b]	2.31 ± 0.13 [b]	2.33 ± 0.35 [a]
IS-CW film wrap/ PE	0.53 ± 0.55 [c]	3.95 ± 0.29 [b]	4.56 ± 0.57 [a]	4.98 ± 0.47 [a]

[a-c] Comparisons are made within the same column means, with different superscripts are significantly different ($P < 0.05$), $n = 3$ for all treatments.

[1] I = whey protein isolate; S = sorbitol; CW = candelilla wax; PE = polyethylene.

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