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MECHANICAL PROPERTIES OF WHEY PROTEIN ISOLATE BASED EDIBLE FILMS AS AFFECTED BY MEAT PROCESSING CONDITIONS AND OPTIMIZATION OF THESE PROPERTIES

presented by

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By

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ABSTRACT

MECHANICAL PROPERTIES OF WHEY PROTEIN ISOLATE BASED EDIBLE FILMS AS AFFECTED BY MEAT PROCESSING CONDITIONS AND OPTIMIZATION OF THESE PROPERTIES

By

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This study was conducted to test the hypothesis that whey protein isolate (WPI)-based edible films could be used as sausage casings comparable to collagen casings. Heat cured (80°C, 24 h or 90°C, 12 h) WPI-based films containing glycerol and candelilla wax were treated under meat processing conditions; the effects of temperature, time and relative humidity on the tensile strength (TS) and percent elongation (%E) of the films were determined. Tensile strength of WPI-based films was lower while %E was similar to that of collagen films. To optimize the mechanical properties of the WPI-based films, different film forming solutions (5.8% or 6.4% w/v solids content), casting on different surfaces (anodized or non-anodized Teflon®), heat curing (90°C, 12 h) and compression molding (single- and double-stage; and varying temperature, pressure and time) were studied. Heat curing and compression molding increased TS and had no effect on %E. In a separate double-stage compression molding experiment, temperature, pressure and time conditions were optimized. No optimum combination of these conditions was obtained. Finally, seal strengths and surface chemistry (using electron spectroscopy for chemical analysis, ESCA) of unsealed and sealed uncured, heat cured and compression molded WPI-based films were studied. Uncured films had the highest seal strengths. Sealed, heat cured and compression molded films had a preponderance of C-O-H and C-O-C bonds, which were not detected in uncured films.

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INTRODUCTION

Edible films have been developed and characterized by several researchers. These films have been developed from various proteins including whey proteins (Mahmoud and Savello 1990; McHugh and Krochta 1994a; Galietta and others 1998; Kim and Ustunol 2001a), casein (lactic-acid and rennet; Chick and Ustunol 1998, Chick and Hernandez 2002), wheat gluten (Micard and others 2000; Larré and others 2000), gelatin (Lim and others 1999), soy protein (Gennadios and Weller 1991; Gennadios and others 1996), egg whites (Gennadios and others 1998a), pea protein (Choi and Han 2001, 2002) and others.

The growing interest in the development and application of edible films has resulted in extensive research focused on the development and characterization of whey protein isolate (WPI)-based films (Kim and Ustunol 2001 a,b; McHugh and Krochta 1994 a,b,c,d; Shaw and others 2002; Sothornvit and Krochta 2001; Sothornvit and others 2003). These films have been found to have good mechanical properties when compared to synthetic polymers such as high density polyethylene and low density polyethylene as well as to other protein based films including soy protein isolate and wheat gluten films. Furthermore, whey protein based films were found to be transparent, bland and flexible, having good aroma, lipid and oxygen barrier properties (McHugh and Krochta 1994 a,b,c,d). Their disadvantage, as with many biopolymers, has been high moisture sensitivity resulting from the hydrophilic nature of the native and denatured proteins.

Applications for whey protein isolate (WPI)-based films have been investigated and suggested including pouches for soups, cocoa powder and coverings for cheese slices

(Amin and others 2001; Kim 2000) as well as anti-microbial casings for hot-dogs (Cagri and others 2003). Kim and Ustunol (2001b) reported that WPI-based films had no distinct milk flavor or odor and were thus acceptable with respect to their sensory attributes. Cagri and others (2003) further confirmed the organoleptic acceptability of the films by demonstrating that hot-dogs made with WPI-based films were as acceptable as hot-dogs made with commercial collagen and natural casings. Findings of the above researchers indicate the potential for WPI-based films to be used in food applications such as sausage casings.

In order for WPI-based films to be used as sausage casings, their mechanical properties need to be comparable to the properties of commercial casings currently used for this purpose. Collagen casings are currently the most widely used edible sausage casings as an alternative to natural casings (Osburn 2002). Brown, caramel and other food grade colors are added to collagen casings to provide products with distinct colors that are marketed as distinctive smoked or spicy specialty products. This practice is often employed because non-colored casings tend to appear gray after steam cooking (Osburn 2002). Heat cured WPI-based films provide the benefit of a natural pigmented appearance similar to caramel or smoked colored casings without the addition of food coloring. Increased yellowness has been reported following heat curing of these films (Kim and others 2002; Cagri and others 2003). These findings further indicate the potential for WPI-based films to be used as sausage casings.

Previous research from our lab has shown that heat-cured WPI-based films have mechanical properties similar to collagen films (Amin and others 2003). A WPI-based

film that can withstand meat process conditions may provide the meat industry with an alternative to collagen films.

The specific objectives of the study were:

(i) To determine if heat cured whey protein isolate (WPI) based edible films could withstand the temperature, time and relative humidity conditions encountered in a meat cooking process scheme used for Polish sausage

(ii) To reformulate the films and optimize their mechanical properties for use as sausage casings.

(iii) To optimize and determine the sealing properties of the WPI-based films developed in the optimized film protocol.

CHAPTER 1

LITERATURE REVIEW

1.1. EDIBLE FILMS

1.1.1. Definition

Edible films are defined as thin layers of edible material able to inhibit migration of moisture, lipids, oxygen, carbon dioxide, and flavor volatiles (Krochta and De Mulder-Johnston 1997). Edible films may be an essential component to quality and stability control of many foods by providing a barrier against mass transfer in foods (Miller and Krochta 1997).

1.2. FORMATION AND STABILITY OF PROTEIN BASED EDIBLE FILMS

Formation and stability of protein based films mostly depends on the formation of cross-links. High temperature, pH extremes – particularly alkaline pH and exposure to oxidizing conditions and enzymatic activity can result in the introduction of protein cross-links or hydrolytic fragments thereby inducing substantial changes in the structure of the proteins. The cross-links are organized according to the amino acids that react to form the cross-links. It is important to note, however, that no matter how extreme the processing conditions may be, not all amino acids will participate in cross-linking (Gerrard 2002).

Some cross-links found in foods are disulfide bonds (S-S), links derived from the Maillard reaction and cross-links formed through transglutaminase catalysis. The

oxidative coupling of two adjacent cysteine residues within a protein forms S-S bonds, the most common and well-characterized cross-links. These bonds are linked together to form polypeptide chains that contribute to a protein's tertiary structure and are important in film formation. A heating process is used to denature the protein, thus unfolding it to reveal free sulfhydryl (S-H) groups. In whey proteins, heating exposes free S-H groups in β-lactoglobulin allowing S-H or S-S interchange reactions to occur (Krochta 1998). Hydrolysis with acid or alkali may also be used to denature proteins. Alkaline conditions aid film formation because S-S bonds are cleaved and reduced to free S-H groups when dispersed in alkaline conditions. Di-sulfide bonds are then reformed during drying of the film forming solutions (McHugh and Krochta 1994c). The S-S bonds resulting from heat denaturation of proteins have been hypothesized to be partly responsible for film structure (Fairley and others, 1996a). In another study Fairley and others (1996b) investigated the mechanical properties and WVP of edible films from WPI treated with N-Ethylmaleimide or cysteine. This study indicated that SH/S-S interchange did not have a significant role in determining the functional properties of WPI-based films. This was evidenced by film retention of WVP and mechanical properties after the blocking of SH/S-S interchange.

Hydrogen bonds are important in stabilization of protein networks. Hydrogen bonds are the electrostatic interaction forces between charged groups of protein molecules and between charged groups on a protein molecule and surrounding water molecules when water is present (Rasco and Zhong 2000). The intermolecular association between the proteins results in brittle films. This is the reason that plasticizers are added to these films as they disrupt some of these associations and thus

decrease the rigidity of the protein structures (Gerrard 2002). Hydrophobic interactions establish another set of interactions between protein groups that are important for the formation of protein networks. These are defined as the attractive forces between non-polar molecules or groups of molecules that induce association of these molecules in an aqueous environment (Stenesh 1989). The hydrophobic groups mainly exist on the inside of the molecules in native proteins; for example, during heating, whey proteins are denatured, hydrophobic groups are exposed and during drying they are drawn closer and intermolecular hydrophobic interactions are established (Cheftel and others 1985).

Since formation and stability of protein-based films depends on the protein used and its amino acid composition, proteins high in cysteine residues will produce films higher in S-S bonds, while those having low cysteine content will have more hydrophobic and hydrogen types of linkages. For example, films derived from corn zein possess more hydrophobic structures than films derived from soy proteins (Krochta 1998). Disulfide bonds have been reported to be responsible for cross-linking in soy-protein based films. These S-S bonds are suggested to predominate in whey protein based films. Native whey proteins are globular proteins containing mostly hydrophobic and S-H groups in the interior of the molecule where they are not readily exposed. Heat denaturation of whey proteins in an aqueous solution has been used for the formation of whey protein films. The three dimensional structure of the protein is altered during heating, exposing the internal SH and hydrophobic groups (Shimada and Cheftel 1998) this further promotes S-S bonding and hydrophobic interaction when the film is subsequently dried (McHugh and Krochta 1994b).

Protein based films are dependent on the protein network formed during protein-protein and protein-solvent interactions as well as the balance between the attractive and repulsive forces between and among polypeptide chains. These structures are referred to as "cross-links" and occur within a protein (intramolecular) and between proteins (intermolecular) (Feeney and Whitaker 1988). Cross-links are vital for maintaining the correct conformation of certain proteins and may control the degree of flexibility of the polypeptide chains (Gerrard 2002). Crosslinking not only stabilizes and aids in film formation but is also important for improving film insolubility properties and tensile strength (Pérez-Gago and Krochta 2001). Functional characteristics of protein-based films are determined by protein-protein interactions. All protein conformational properties may be dictated by the primary structure of the native protein (amino acid sequence). Thus, film forming ability may be influenced by amino-acid composition, distribution and polarity; conditions affecting formation of ionic cross-links between amino and carboxyl groups; presence of hydrogen-bonding groups and intramolecular and intermolecular S-S bonds (Gennadios and Weller 1991).

1.3. PROPERTIES OF PROTEIN BASED FILMS

Edible films are often studied to determine their ability to protect foods from the environment and adjacent ingredients. Film water vapor permeability, oxygen permeability, aroma and oil permeability are important for many foods. Mechanical properties including tensile strength (TS) and elongation (%E) are most commonly investigated to assess the film's ability to protect foods from mechanical abuse (Krochta 1997).

1.3.1. Barrier properties

1.3.1.1. Water vapor permeability

This is the most commonly studied property of edible polymer films. Due to the hydrophilic nature of proteins, their moisture barrier properties tend to be relatively low. The water vapor permeability (WVP) of edible films is affected by relative humidity (RH) and plasticizer type and amount as well as lipid type and amount when lipids are added (McHugh and Krochta 1994 a,c; Shellhammer and Krochta 1997). When compared to synthetic materials or polymers such as cellophane and low density polyethylene (LDPE), plasticized whey protein films have a WVP ranging from 1-4 orders of magnitude greater than cellophane and LDPE, respectively (Morillon and others 2000). This indicates that WPI-based films need to be optimized so that their WVP can be reduced to increase their potential for use in increased RH conditions.

To decrease WVP, the hydrophobicity of the films can be increased. This has been done by either: 1) addition of a lipid to the film forming solution to produce emulsion composite or 2) laminating the film with a lipid layer. Lamination processed films were found to have significantly improved water vapor barrier properties compared to emulsion formed films (Weller and others 1998; Cho and others 2002). Laminating, however, requires more steps than forming a composite film in which both hydrophilic and hydrophobic components are dispersed in the film forming suspension. Furthermore, delamination of these films due to the high surface energy existing between the polar and nonpolar materials can be a problem (Kamper and Fennema 1984; Shellhammer and Krochta 1997). McHugh and Krochta (1994c) further reported that decreasing the mean

droplet diameter of film forming emulsions correlated well with a linear decrease in WVP.

More success has been found in homogenizing the lipid into the protein film forming solution to form emulsion-composite films. This is intricately enhanced by the emulsifying ability of whey proteins (McHugh and Krochta 1994a; Shellhammer and Krochta 1997; Perez-Gago and Krochta 1999). Lipids such as fatty alcohols and acetylated monoacylglycerols increase WVP of whey protein films while beeswax, carnauba wax, candelilla wax (CW) and paraffin wax, which all possess hydrophobic characteristics, contribute to improved resistance of films to water vapor permeability. Studies by Gontard and others (1994) concluded that the use of lipids with higher melting points resulted in decreased WVP of wheat gluten (WG)-lipid films. Chick and Hernandez (2002) studied the effect of increasing wax (carnauba or candelilla) content on the WVP of lactic-acid casein based films. Wax content was increased from 5 to 15% (w/w of protein) and WVP was tested for all wax levels at two RH conditions: 50% and 70%. At 70% RH carnauba wax did not effectively decrease WVP despite increases in wax content. They indicated that CW was more effective than carnauba wax in reducing WVP of lactic acid casein-based films at increased RH.

1.3.1.2. Oxygen permeability

Oxygen permeability (OP) for protein films such as whey protein isolate (WPI), soy protein isolate (SPI) and lactic-acid casein has been reported to be relatively high and comparable to Nylon 6 films (Chick 1998). The addition of lipids such as beeswax, acetylated monoacylglycerol and microcrystalline wax has been shown to increase the OP of protein films (Gennadios and others 1993b; Donhowe and Fennema 1993). The

increase in OP after the addition of microcrystalline wax was attributed to the microscopic sized crystals creating intercrystalline pathways for oxygen permeation. The presence of irregular crystal sizes and lattice distortions were given as the reason that lead to these intercrystalline pathways (Donhowe and Fennema, 1993).

Studies by McHugh and Krochta (1994d) on the effect of plasticizer type and concentration (15-30% w/w of protein for glycerol and 30-50% w/w of protein for sorbitol) on OP of WPI-based films showed that films containing sorbitol provided better oxygen barrier properties than those containing glycerol at equal concentrations (30%). They also showed that as plasticizer concentration increased, OP increased for both plasticizers (4.3 to 11.6 $cm^3\mu m/m^2.d.kPa$ and 18.5 to 76.1 $cm^3.\mu m/m^2.d.kPa$ for sorbitol and glycerol plasticized films respectively). Plasticizer content increased from 35-50% (glycerol) and 15-30% (sorbitol). These results were for films tested at 50% RH. When WPI-based films were compared to the synthetic polymers high density polyethylene (HDPE) and ethylene vinyl alcohol (EVOH), they showed lower OP than HDPE and compared favorably with EVOH, making the use of these films (WPI-based) in control of oxidation and respiration in food systems extremely promising. In experiments conducted by Chick and Hernandez (2002) on lactic-acid-casein-based films, the addition of lipid (carnauba or candelilla wax) and lipid concentration (5-15% w/w of protein) were found to have no significant effect on OP. Increase in RH from 0-50% had no significant effect on OP, but as RH increased from 50% to 70%, significant increases in OP were observed. These were: 0.63 to 2.21 MPa and 0.76 to 1.12 $m^3.m/m^2s.Pa$ for carnauba and candelilla wax (CW) respectively. These researchers made comparisons to polyester and

showed that protein films had better OP than polyester (0.63 and 0.76 $m^3.m/m^2.s.Pa$ for carnauba and CW containing films vs. 3.24 $m^3.m/m^2.s.Pa$ for polyester films at 50% RH).

Kim (2000) reported a significant decrease in OP in emulsion films made with WPI or whey protein concentrate (WPC) and CW compared to films without wax. Increasing wax content, however, did not further decrease OP. Films containing 2, 4 or 8% wax (w/w of protein) were reported as having no significant differences in OP. Unlike the relationship observed for WVP, OP did not conform to predictable trends associated with formulation or film formation procedures.

1.3.2. Mechanical properties

Mechanical properties possessed by a polymeric material are determined by the polymer's stress-strain tensile characteristics. Stress is measured as the force/area and strain is the dimensionless fractional increase in length (Hernandez 1997). The ultimate tensile strength (TS) is the maximum tensile stress a material can sustain prior to rupture. Ultimate elongation (%E) is the strain at which the sample breaks (Hernandez 1997).

1.3.2.1. Factors affecting mechanical properties

There are several factors that affect mechanical properties of edible protein films. Gennadios and others (1993a) found that TS of WG and soy protein isolate (SPI) films increased as pH increased above the isoelectric point (pI) of the proteins. For soy protein films the optimum pH range was 6-11; both TS and %E of SPI-based films were found to peak within this pH range. At acidic pH (1-3), TS and %E were significantly lower than at pH 6-11. For WG films however, differences in %E at high and low pH were not significant. Since S-H reactivity increases at pH > 8, film formation is favored by more alkaline pH (Banerjee and Chen 1995). Extreme pH (acidic or alkaline), however, may

result in reduced TS and %E (Gennadios and others 1993a). The reduced mechanical properties would be due to the fact that as pH shifts away from the pI, protein molecules repel one another and protein-protein interactions are reduced as the protein becomes more soluble. For the film to have adequate mechanical properties, protein-protein interactions are required that become reduced when the protein is very soluble.

McHugh and Krochta (1994d) studied the effect of plasticizer type and amount on mechanical properties of WPI-based edible films. They reported that increasing glycerol and sorbitol significantly decreased TS while increasing %E. Levels of glycerol studied were 15% and 30% (w/w of protein), TS was found to be the same for equal sorbitol and glycerol concentration (30%). As plasticizer concentration increased, TS of glycerol plasticized films decreased more than that of sorbitol plasticized films. However, %E of glycerol plasticized films exhibited higher elongation than sorbitol plasticized films prepared at 30% plasticizer concentration. Sothornvit and Krochta (2001) also reported that plasticizer type and amount affected mechanical properties of β-lactoglobulin (β-lg) films. These researchers studied the effect of glycerol, polyethylene glycol (with molecular weights of 200 = PEG 200 and 400 = PEG 400), sorbitol, sucrose and propylene glycol at 0.34, 0.44, 0.54 and 0.64 M concentrations. Glycerol and PEG 200 were the most effective plasticizers providing the best mechanical properties. A linear increasing relationship was found between %E and plasticizer concentration; these increases in plasticizer concentration, however, reduced TS.

Experiments by Fang and others (2002) showed that the effect of protein concentration on mechanical properties of WPI films was not as significant as that of plasticizer concentration. They studied the effect of increasing β-lactoglobulin (β-lg)

from 40% to 90% while keeping the total weight of protein constant at 12% w/w of the solution. Glycerol was added at a concentration of 20% or 40% to all protein solutions (protein to glycerol rations of 4:1 or 3:2, respectively). Films prepared with 20% glycerol had higher TS than films possessing 40% glycerol. Increase in protein concentration did not increase TS. The %E of films plasticized with 20% glycerol was lower than that of films plasticized with 40% glycerol; again the protein concentration had no effect on %E. Possible increases in TS resulting from increased protein content may not have been observed due to the high plasticizer concentrations used.

The effects of beeswax, CW, carnauba wax and a high temperature melting fraction of anhydrous milk fat on TS and %E of whey protein based films were investigated by Shellhammer and Krochta (1997). They found that increasing lipid levels decreased TS for all lipid types. Films formulated with carnauba wax were the strongest of all the films at all lipid levels tested. Elongation was not significantly affected by the lipid type or concentration except for milk-fat-added films. Increase in the concentration of milk fat increased %E significantly, this could have been due to plasticizing effects of unsaturated and low molecular weight triacylglycerols in the milk fat. Lipid type and concentration were important factors affecting mechanical properties of the protein-lipid emulsion films. Contrary to these findings, Kim (2000) found a significant increase in the TS of WPI-CW emulsion films as wax content increased from 0 to 8% (w/w of protein). Whey protein concentrate (WPC)-CW emulsion films showed significant increase in TS with the addition of wax, but, as wax content increased from 2 to 8% (w/w of protein), no further increases in TS were observed. The addition of CW to WPC films decreased %E, however this decrease remained unchanged as wax content increased.

1.3.2.2. Means of improving mechanical properties

Mechanical properties of protein-based films can be improved by cross-linking of proteins. Cross-links confer elastomeric properties due to the formation of intermolecular covalent linkages (branched chains). These branches increase the rigidity of a material. When the cross-linking density is sufficiently high, it increases the water resistance of the film (Gontard and others 1994). There are several ways to achieve this including enzymatic, physical and chemical means. Transglutaminase is an enzyme that has been used in the cross-linking of proteins (Mahmoud and Savello 1990, 1993; Lim and others 1999). Transglutaminase catalyzes acyl-transfer reactions that cause formation of glutaminyl-lysine intra and intermolecular cross-links in protein (Nielsen 1995). Larré and others (2000) reported that transglutaminase increased both TS and %E in wheat gluten films. Enzymes, however, are generally costly and this limits their application on large scale (Vachon and others 2000).

Gamma and ultraviolet (UV) irradiation have been used as physical means to cross-link protein films (Ressouany and others 1998; Brault and others 1997). Brault and others (1997) reported increased resistance to puncture and moisture due to irradiation treatment of caseinate films. Maximum mechanical strength of irradiated caseinate films was obtained at 64 kGy (Ressouany and others 1998). Vachon and others (2000) also reported that γ-irradiation was efficient in inducing cross-linking of WPI-caseinate films. WPI (5% w/w) film forming solutions were irradiated at a total dose of 32 kGy following heating of the solutions at 90°C for 30 min. Evidence of the cross-linking was shown by an increase in molecular mass from 0.2 x 10^3 to >10 x 10^4 kDa when compared to a native protein solution. The lower dosage required for these films was attributed to a

higher dose rate [38.1 kGy/h vs. 1.5 kGy/h used by Ressouany and others (1998)]. Ultraviolet (UV) radiation treatment has also been used in cross-linking of sodium caseinate films (Rhim and others 1999) and SPI-based films (Gennadios and others 1998b; Rhim and others 2000). In both studies, UV treatment resulted in decreased puncture strength (PS), increased TS and decreased %E. The researchers suggested that UV treatment resulted in the occurrence of other covalent bonds other than S-S bonds. Although UV treatment decreased the solubility of these of films, there were no differences in TS compared to the untreated control. The effect of UV irradiation in WPI-based edible films was reported by Lim and others (1999). They demonstrated that UV radiation reduced water solubility and increased TS of these films.

Another physical means of improving properties of protein based edible films is heat curing. Heat curing involves application of heat to polymers to induce cross-linking. Gennadios and others (1996) reported that heat curing soy protein films at 80 or 90°C resulted in films with increased TS, reduced %E, increased moisture content and WVP. Miller and others (1997) reported similar results with heat cured whey protein films. They suggested that heat curing might elicit additional cross-linking of proteins. Rhim and others (2000) also investigated heat-curing of SPI films. Film strips were heat-cured at 90°C for 24 h in an air-circulating oven. Heat-curing increased TS from 8.2 ± 0.2 MPa to 14.7 ± 0.4 MPa. %E was reduced from 30 ± 3.3 to 6.1 ± 0.7%.

Chemical agents used for covalent cross-linking of proteins include gluteraldehyde, glyceraldehydes, formaldehyde, glyoxal and lactic acid. Galietta and others (1998) reported that cross-linking of whey proteins using formaldehyde enhanced mechanical properties and decreased solubility of whey protein films. Gluteraldehyde is

commonly used as a cross-linking agent in the production of collagen casings (Osburn 2002). Although the gluteraldehyde is chemically reacted with the collagen so that it should not be available in its free form, the toxicity of gluteraldehyde in these materials is still of concern as they may have residual gluteraldehyde. Dialdehyde starch and 1-1'carbonyldiimidazole have been investigated as less toxic alternatives to the toxic aldehyde cross-linkers mentioned above (Gennadios and others 1998a). Furthermore, cross-linking of casein with gluteraldehyde has been effectively used to create a high definition matrix suitable for controlled drug release applications (Latha and Jayakrishnan, 1994; Lanaerts and others 1991).

Fang and others (2002) also studied the effect of calcium chloride (Ca^{2+}) content on the mechanical properties of WPI-based films. Films containing 20% glycerol (4:1 protein:glycerol) to film forming solutions demonstrated increased TS following the addition of 10 mM Ca^{2+}. The higher ionic strength due to Ca^{2+} at this concentration may have shielded electrostatic repulsions encouraging protein-protein interactions, which resulted in greater TS. However, TS of WPI films prepared with 40% glycerol (3:2 protein:glycerol) only slightly increased after the addition of 5mM Ca^{2+} to film forming solutions. It is noteworthy that the addition of 10mM Ca^{2+} resulted in fragile films that could not be tested for mechanical properties.

The addition of calcium chloride ($CaCl_2$; 0.04% w/v)) into WPI film forming solution as a cross-linking agent used to improve mechanical and water vapor barrier properties was also reported by Cagri and others (2001). The rationale used was the phenomenon that the divalent cation, Ca^{2+}, cross-links between negatively charged groups on proteins and thus increases cohesion between protein chains, reducing protein

polymer segmental mobility and improving both mechanical properties and WVP (Krochta and others 1990). It was also stated that the addition of Ca^{2+} enhanced protein aggregation at low pH (<6.5).

Park and others (2001) investigated the effect of calcium salts and glucano-δ-lactone (GDL) on mechanical and moisture barrier properties of soy protein isolate (SPI) based films. The levels of calcium salts and GDL used were 0.1, 0.2 or 0.3% (w/w of SPI). Their results showed that $CaSO_4$ improved TS and puncture strength (PS) while $CaCl_2$ did not result in a significant improvement in these properties. Protein solubility enabled divalent Ca^{2+} to bind strongly with polar groups on protein to form denser 3-dimensional networks in SPI films. Calcium salts also improved WVP of the SPI-based films. $CaSO_4$ reacted with protein constituents to yield insoluble protein fragments and altered film consistency; calcium bridges maximized interactions between negatively charged molecules and improved protein network and stability. GDL was also effective in improving mechanical and moisture barrier properties as it increased hydrophobicity and decreased solubility of the unfolded protein and thus promoted its aggregation.

The addition of calcium to whey proteins in film forming solutions presents a possibility for developing cross-links through the addition of negatively charged ions. However, no studies were found in the literature that investigated this hypothesis.

1.3.3. Moisture sorption isotherms

Moisture sorption isotherms (MSI) are plots of correspondent equilibrium moisture content (EMC) of a material with its water activity (a_w) at a constant temperature. Water activity is also used in reference to RH. Moisture sorption isotherms provide a valid indicator of a food's storage stability in reduced moisture environments;

they also characterize the status of water (bound or free) in the food (Coupland and others 2000). Moisture sorption isotherms of food products must be determined to understand the behavior of the food product in different moisture or RH environments. They are especially important for edible films if they are to be used to protect foods from moisture transfer.

Coupland and others (2000) studied the effect of glycerol on moisture sorption of WPI-based films. Glycerol was added in the following ratio: [glycerol (G):non-volatile material (NVM)] where NVM = glycerol and WPI. The ratio of G:NVM was expressed within the range of G = 0-0.5. They found that the moisture sorption of WPI-based films increased slowly as RH increased up to a_w ~ 0.75 after which it increased rapidly. Films higher in glycerol content had higher moisture sorption; native WPI powder had the same sorption characteristics as WPI-based films without glycerol. These results showed that denaturation and film casting had no effect on the protein's capacity to bind water.

Chick and Hernandez (2002) reported on the MSI of lactic-acid-casein films plasticized with sorbitol (3.5, 4.0, 4.5, or 5.0% w/w) and containing CW or carnauba wax (5, 10 or 15% w/w of protein). The films had low moisture uptake up to 70% RH but this rapidly increased after 85% RH. RH was shown to decrease TS of the films; at 75% RH, films possessed 5-fold less strength than at 50% RH. The results also showed an increase in %E following an increase in RH within the same wax level.

Similar results were reported by Cho and Rhee (2002) for SPI films plasticized with glycerol, sorbitol and a 1:1 mixture of glycerol and sorbitol. Plasticizer was added at three levels (0.3, 0.5 and 0.7 plasticizer/g SPI). They found that films with higher plasticizer content had higher EMC and films higher in glycerol ratio absorbed more

moisture. It was suggested that this phenomenon resulted because glycerol has higher moisture affinity than sorbitol. Yoshida and others (2002) weighed pieces of films placed at 75% RH over a fixed period to determine weight gain. They showed that adsorption of moisture for whey protein films followed a linear diffusion model by.

Investigations by Kim and Ustunol (2001a) showed that EMC of WPI-based films plasticized with glycerol increased slowly up to a_w of 0.65 after which EMC rapidly increased. The presence of CW reduced the amount of moisture uptake. Films were compared to WPI-based films plasticized with sorbitol. Glycerol-plasticized films resulted in a higher EMC than sorbitol plasticized films; this was attributed to the hydroscopicity of glycerol. Their results indicated that EMC appeared to be a function of the plasticizer hydroscopicity. Further research is required to determine how WPI-based films would function in a high RH environment via practical tests under these environmental conditions. Additionally, the films tested by Kim and Ustunol (2001a) were uncured films; the effect of RH on cured WPI-based films requires investigation.

1.3.4. Thermal and sealing properties

Thermal characteristics provide information necessary for determining processing parameters for edible films. Three temperatures are frequently reported with respect to polymers: the glass transition temperature (T_g), the temperature at which reversible change occurs in amorphous polymers or in amorphous regions of a partially crystalline polymer (Hernandez 1997); the melting temperature (T_m) and the degradation temperature (T_d). T_m is the solid to liquid phase change temperature while T_d is the point of thermal disruption of bonds in the absence of oxygen

Using thermogravimetric analysis, Ogale and others (2000) studied the thermal characteristics of SPI films plasticized with glycerol (30 w/w). They determined the upper thermal processing limit to be 150°C. Rapid degradation was observed at temperatures above 180°C for plasticized films. Non-plasticized films degraded at 200°C. Similar processing temperatures were reported by Cunningham and others (2000) for SPI films.

Thermal properties of lactic-acid casein were studied by Chick and Hernandez (2002). Differential scanning calorimetry (DSC) results showed that lactic-acid-casein powder melts between 100 and 110°C. Candelilla wax, a component often used to increase hydrophobicity of edible films, had a narrow endothermic peak (68-73°C). Lactic-acid-casein-sorbitol films had a peak in the temperature range 100-110°C. Decomposition of lactic-acid-casein films started to occur at 120°C.

WPI-based edible film thermal characteristics were determined by Kim and Ustunol (2001c). The composite film (WPI-glycerol-CW) had a transition onset temperature of 108°C and transition peak at 145°C. These characteristics were used to determine optimal sealing conditions for WPI-based composite films. Stehling and Meka (1994) reported on the effect of melting distribution on heat-sealing properties of polyolefins. Melting distribution was important for seal strength determination and they reported that DSC analysis could be used to accurately determine the melting distribution and hence establish the heat sealing curve of the film.

Sealability of edible films is an important attribute as it determines the possibility of using these films in food applications such as casings for sausages. Sealing involves melting of specified polymer regions by the application of heat and bringing them

together while they are in a partially molten state to form integrated bonds between the two polymer surfaces. Pressure is applied causing polymer chain segments on opposite sides of the interface to diffuse across the interface. Thus, molecular entanglements are induced between the melted surfaces and fused upon cooling (Meka and Stehling 1994).

Seal formation depends on the sealing temperature, applied pressure (the force applied during sealing) and dwell time (the length of time the sealing bars are kept in contact during sealing). Optimization of dwell time is an important control point. Dwell times need to be carefully monitored because sealing at high temperatures for a short time may result in poor temperature control, conversely long dwell times should also be avoided as they may result in distorted seals. The surface must be pressed together an adequate length of time and with adequate pressure to induce diffusion across the interface and the formation of bridged bonds (Mueller and others 1998).

Appropriate seal temperature is determined by examining thermal properties of the polymer to be sealed. Thermal properties and heat-sealing conditions of WPI-CW edible films were investigated by Kim and Ustunol (2001c). Whey protein isolate based edible films were heat-sealed on the non-lipid oriented side of the film because the lipid-oriented sides did not adhere well. Heat sealing temperatures were based on onset thermal temperature (T_o) of WPI-CW emulsion films as determined by DSC analysis. The seal strengths for glycerol and sorbitol plasticized films containing CW ranged from 141 ± 36 to 297 ± 15 N/m for glycerol-plasticized films and 105±0.9 to 296 ±0.6 N/m for sorbitol-plasticized films. The highest seal strength corresponded with the T_o (108-122°C) for glycerol-plasticized films and (126-127°C) for sorbitol plasticized films. It was suggested that these results might be used to determine thermal processing

temperatures for WPI-based edible films. The lowest seal strengths for glycerol and sorbitol plasticized films were obtained when the films were heat-sealed at 130°C and 110°C respectively. The lower seal strengths at higher temperatures for the glycerol-plasticized films were explained as having resulted from excessive temperature that caused distorted and weaker seals. According to Martin (1986), when the heat required to produce a seal exceeds the heat-sealing temperature range for that material, it induces distorted or non-functional seals. Based on the results of Kim and Ustunol (2001c) it was recommended that heat-sealing temperatures should not exceed 130°C for glycerol plasticized WPI-based films.

Results obtained by Kim and Ustunol (2001c) for sorbitol plasticized films were comparable to seal strengths obtained by Chick (1998) for lactic acid casein films plasticized with sorbitol (153-247 N/m). Experimental conditions for these studies, however, were not identical even though both researchers used a thermal sealer. Seal temperatures used by Chick ranged from 93°C to 121°C at a pressure of 410 kPa. Seals formed using a voltage based impulse sealer have not been reported. Because of the broad surface area required to form seals with the thermal sealer, which may range up to 1.5 cm seal widths, voltage-based impulse sealing might be more appropriate in food applications since more narrow seal widths can be obtained (0.25 cm). This would be an advantage since less area and total film would be used.

1.4. ELECTRON SPECTROSCOPY FOR CHEMICAL ANALYSIS

Seal strength values provide general information about the strength of the bond; this information, however, does not provide insight into the chemical characteristics of

the bond. By conducting a surface analysis, surface specific information is obtained that can be valuable for determining chemical changes due to sealing. This information can also be useful when developing new materials and applications (Rindlav-Westling and Gatenholm 2003). Electron Spectroscopy for Chemical Analysis (ESCA), also referred to as XPS (X-ray photoelectron spectroscopy) provides insight into the surface chemistry of the material tested and has been used as the principal technique for defining interfacial molecular properties associated with adhesive behavior of materials. The use of ESCA allows identification and quantification of all elements except hydrogen and helium (Ratner and Castner 1997). It is an ideal technique to study surfaces of sealed films because of its surface sensitivity (Gerenser 1993).

Surface composition of starch, amylose and amylopectin films was investigated by Rindlav-Westling and Gatenholm (2003) using ESCA. Resolved C1s spectra showed the presence of nitrogen which was believed to have been derived from protein, bonds observed were O-C=O, N-C=O, O-C-O and C-C. These researchers stated that C-C bonds did not originate from the starch in the polymer but from proteins, lipids and possibly contaminants on the sample surface. Kim and Ustunol (2001c) conducted a unique study where seals of uncured whey protein based films were investigated. Findings of this research showed that carbon was the main element of the films followed sequentially by oxygen and nitrogen. After heat-sealing, there was a reduction in carbon by 1.4 - 6.5% depending on the film tested. However, oxygen and nitrogen increased by 1-2% and 1-4% respectively under these sealing conditions. Kim and Ustunol (2001c) suggested that that some oxygen and nitrogen components had formed on the surface. It was, however, not explained how this might have happened. Possibly, by virtue of the

decrease in C and moisture loss there was a subsequent or apparent increase in these elements. High-resolution ESCA peaks showed C1s, O1s and N1s spectra. Kim and Ustunol (2001c) suggested that formation of C-O-H and N-C bonds upon heat sealing might be responsible for the mechanism of seal formation. These data provided by these researchers focused on uncured WPI-based films; properties of cured WPI-based films have not been investigated using this method.

1.5. COMPRESSION MOLDING

Compression molding is a thermal process that was originally developed by the automobile industry for replacement of metal components with composite parts. The molding process can be carried out for either thermoset materials or thermoplastics. Most applications today use thermoset polymers, and compression molding has become the most common processing method employed (Davis and others 2003).

The advantages of compression molding include short cycle times, typically running at 1-6 minutes, high volume production and high quality surfaces. Disadvantages include high capital investment, labor intensiveness, and secondary operations that are at times required (Davis and others 2003). The advantage of short cycle times involved in using compression molding has been maximized in developing edible WPI-based films. Using compression molding, Sothornvit and others (2003) developed WPI-based films within only 2-3 minutes. This proved to be an advantage over the solvent casting method that takes 18-24 hours, excluding preparation time.

More recently, compression molding has been applied to the production of plastic sheets including biodegradable sheets made from soy and wheat protein (Cunningham and others 2000; Mo and others 1999; Mo and Sun 2000; Zhang and others 2001).

Production of SPI plastic sheets has received commercial interest due to the abundance and relative low cost of the soy bean, and particularly due to environmental considerations (biodegradability/renewable resource) (Wu and Zhang 2001). Wu and Zhang (2001) applied compression molding to the development of SPI films in which the properties and structure of SPI-ethylene glycol (EG) sheets were investigated. Films were compression molded at 150°C with 15 MPa of pressure for 1 min. The sheets formed had good TS, %E, water resistance and thermostability (films containing 50% g/100g of EG) had a TS of 4.23 MPa, %E of 220% and water resistance was higher than that of thermoplastic starch or cellulose film.

Cunningham and others (2000) studied the tensile properties of SPI films formed through compression molding. Films were made with various plasticizer amounts (20, 25, 30, 35, 40% w/w of protein) and variations in mixing procedures and aging (1 week or 4 weeks) of the films prior to testing were employed. Aging consisted of keeping the protein mixture for 1 or 4 weeks before developing films. Aging conditions (temperature and relative humidity), however, were not specified. Thickness and tensile strength values ranged from 0.16 mm and 1.6 MPa at the highest plasticizer level (40 w/w) to about 0.35mm and 15.8 MPa at the lowest plasticizer content (20 w/w). Compression molding conditions were established as: 150°C, 10 MPa for 2 min. Elongation (%) ranged from 1.5% to 106% and increased with increasing plasticizer content. In a companion study, Ogale and others (2000) determined the processing temperature for SPI based compression molded films to be about 150°C; thermal degradation temperatures were determined to occur above 180°C.

Very limited research has been conducted on the compression molding of WPI films; yet, like soy proteins, these proteins have received much interest for potential use in food industry applications. Sothornvit and others (2003) determined the effects of moisture and glycerol content on film WVP and protein solubility, using film obtained by compression molding. The pressures studied ranged from 0.81MPa – 2.25 MPa. Compression molding was conducted for 2 minutes because at high temperatures films degraded after 2 minutes. According to Sothornvit and others (2003), the feasible temperature range for compression molding of WPI-based films was determined to be 104°C – 140°C. Film formation without degradation was obtained at temperatures lower than 140°C. Temperatures within this range should provide ideal conditions for studying combinations of time, temperature and pressure for WPI films as they fall within the range of temperatures recommended by Kim and Ustunol (2001c) for thermal processing of WPI films. No research has been conducted using compression molding as a post treatment for solvent cast films. Research into the possibility of this application is required.

1.6. COLLAGEN CASINGS AND SAUSAGE MANUFACTURE

Casings are important in the sausage manufacturing industry and are used for forming sausage products into a specific shape and or portion control. Different types of casings are used and these include natural casings, reconstituted or regenerated collagen casings and regenerated cellulose casings (Wang 1986). Traditionally, natural casings from beef, pork and lamb were stuffed with comminuted meat. However, high bacterial loads, preservation requirements (salting or salting and refrigeration), absence of

uniformity (thickness, color and diameter) and breakage limited utility of these casings. These are inherent problems with the use of natural gut casings, which are further complicated by the labor intensive process of cleaning these casings. Furthermore, the performance and supply of natural casings hindered the processed meat industry's ability to move forward into modern automated sausage manufacture at higher production capacities These factors led to the development of regenerated collagen casings. (Osburn 2002).

Collagen casings are primarily produced from cattle hides. The hides are split in half and collagen is obtained from the corium (flesh side) of the skin. They may be manufactured in both edible and non-edible varieties. The edible varieties are smaller, thin, and chewable and solubilize during cooking. Hood (1987) and Courts (1977) describe two different methods used for the industrial manufacture of collagen casings, the *'dry'* and *'wet'* methods. The *'dry process'*, also called "dry spinning technology" was developed in Europe during the 1930s and results in a high solid content suitable for extrusion.

The *'wet process'* also called "wet spinning technology" consists of using the hide corium from acid- or alkaline-dehaired cattle hides which are decalcified, ground into small pieces and then mixed with acid to produce a viscous suspension (4-5% solids) which is pumped through high shear homogenization. Cellulose and carboxymethylcellulose may be added to improve mechanical properties of the casing. The acidified collagen slurry is then extruded to form a woven fiber structure, passed through a coagulation bath of brine and then formed into a tubular casing shape. The high salt concentration and acid neutralization shrinks the collagen fibers to form a strong

casing. The casings are then washed to remove the salt and treated with plasticizing (glycerol and sorbitol) and cross-linking (gluteraldehyde) agents to improve casing strength and pliability. The plasticized collagen casings are then collapsed in an "accordion-like fashion" (shirred) so that they can fit over various sized sausage-stuffing horns. The collagen casings are then dried under special temperature and humidity conditions, to 13–18% moisture content, before they are sealed in plastic bags and packed in boxes. The shelf-life of these products depends upon maintaining favorable (cool and dry) storage conditions (Hood 1987).

Collagen casings must possess a variety of product attributes to aid in the manufacturing of various sausage products with numerous types of sausage manufacturing equipment. Casing strength is therefore important for withstanding the rigors of high speed filling and linking operations. The strength of casings is affected by solids content (fiber content), drying conditions, and addition of cross-linking agents such as salts and enzymes. Another means for strengthening collagen casings is UV radiation. Collagen casings may be colored to enhance the external color and appeal of various sausage products. Colors available include blush, brown, red and smoke. Smoke colored collagen casings may be used to replace natural or liquid smoke application or reduce quantities necessary for uniform smoke uptake. Smoke-colored casings provide a richer uniform smoke-colored product surface (Osburn 2002).

The term sausage refers to chopped or minced meat preserved by salting. Traditionally, the mixture would be encased in animal intestines or stomachs. As a result, the characteristic shape of the sausage was cylindrical. The definition of sausage is derived from these properties; that is, sausages are salted, seasoned and chopped meat

products that are generally cylindrical in shape (Pearson 1984). Sausages are normally manufactured from the less expensive cuts of meat and from byproducts; hence they are economical. Process variables associated with final product quality can be classified into several groups: 1) degree of chopping and added water; 2) amount of cooking, smoking and curing; and 3) fermentation time and final moisture of the product.

Cooked sausages are comminuted, semi-solid sausages prepared from one or more kinds of raw skeletal meat and/or poultry meat. Generally, they contain no more than 30% fat and 10% added water and may or may not be smoked. There are three basic types of smoking/cooking methods: 1) natural air circulation 2) air-conditioned or forced air and 3) continuous. When cooking is carried out simultaneously with smoking, temperature and RH are very important. The cooking is often achieved by gas or steam heat. The RH is usually highest at the highest temperature because heat transfer is more efficient at higher RHs. Target temperatures in the final processing stage are expressed as internal temperature reached by the product (Pearson 1996).

Smoke uptake, also referred to as deposition of the smoke on the meat, is dependent upon smoke density, smokehouse air velocity, smokehouse RH and the surface area of the product. RH affects the rate of deposition as well as the nature of the deposit. Higher RH results in more smoke deposition but reduced color development. The smoking/cooking process must therefore be carefully monitored to produce required or desired results (Pearson 1996).

CHAPTER 2

EFFECT OF MEAT PROCESSING CONDITIONS ON MECHANICAL PROPERTIES OF HEAT CURED WHEY PROTEIN-BASED EDIBLE FILMS: A COMPARISON TO COMMERCIAL COLLAGEN CASINGS

2.1. ABSTRACT

Edible films were produced using WPI (5%, w/w), glycerol (3.3%, w/w) and candelilla wax (CW; 0.8%, w/w). One set of films was heat cured at 90°C for 12 h and another at 80°C for 24 h. The WPI-based films together with collagen films were put through a meat processing scheme typical of Polish sausage manufacture. The meat processing conditions were *stage 1*: 57°C/60min/36%RH; *stage 2*: 65°C/90min/60%RH; and *stage 3*: 77°C/30min/80%RH. The effects of meat processing conditions on mechanical properties: tensile strength (TS) and elongation (%E) were determined. All films remained intact throughout the process. Initially TS of WPI-based films heat cured at 90°C for 12 hours was similar to that of collagen films ($p<0.05$) but decreased during the second stage of the cooking process. The TS of films heat cured at 80°C for 24 hours was lower ($p<0.05$) than that of collagen films before processing and also decreased during the cooking process. The %E of collagen films and WPI-based films were similar before and after processing.

2.2. INTRODUCTION

Various applications of whey protein isolate (WPI)-based films have been proposed, such as wrapping for cheese slices, individual serving pouches for cocoa mix (Kim 2000) and antimicrobial sausage casings for hot-dogs (Cagri and others 2003). Sensory evaluation of WPI-based films showed that the films had acceptable taste, and no distinct milk flavor or odor was detected in them (Kim 2000). Cagri and others (2003) developed antimicrobial WPI-based films with which they made hotdog casings. They conducted a sensory evaluation of the hotdogs and demonstrated that the casings were of acceptable taste and color in comparison to commercial collagen and natural casings. Their findings indicate the potential for WPI-based films to be used in food applications such as sausage casings.

Sausage manufacture involves the use of high relative humidity (RH) for the purpose of heat transfer to the core of the sausage product (Ockerman 1989). The meat product in contact with the casing is also high in moisture, beef and pork contain between 70 and 73% moisture content and sausage formulations have water added that my increase the moisture content to ~99%. The hydrophilic nature of proteins presents a challenge in using the WPI-based films in high moisture environments. Several researchers have shown that with increased relative humidity (RH), the moisture absorption of protein-based films, specifically WPI-based films dramatically increases. This increase results in increased elongation (%E) and decreased tensile strength (TS) (Coupland and others 2000; Kim and Ustunol 2001a; Chick and Hernandez 2002). The mechanical properties (TS and %E) of edible films are important in determining their application to foods because these properties provide an indication of how much stress

and strain the film can withstand prior to fracture (Hernandez 1997). This is important because sausage casings must be tender enough to be pliable during stuffing and yet be strong enough to hold the meat batter during cooking (Osburn 2002). The hydrophobicity of films has been increased by the addition of lipids. Kim and Ustunol (2001a) studied the effect of candelilla wax (CW) and butterfat on film properties and showed reduced moisture sorption and increased TS with the addition of CW. Chick and Hernandez (2002) studied the effect of carnauba wax and CW on the barrier and mechanical properties of lactic-acid-casein films. Their findings revealed that the addition of wax, particularly CW, not only decreased water vapor permeability of the films but also increased TS compared to films without wax. To further improve the tensile properties of protein-based films, various methods have been used. Tensile strength has been optimized through heat curing, among other physical or chemical means. Heat curing of WPI-based films was reported to increase covalent cross-linking and thus cause increase in TS and reduce %E (Miller and others 1997). Similar results were reported for soy protein isolate (SPI) films (Gennadios and others 1996; Rhim and others 2000).

In order for WPI-based films to be used as sausage casings, their mechanical properties need to be comparable to the properties of commercial casings currently used for this purpose. Collagen casings are currently the most widely used edible sausage casings as alternatives to natural casings. Sausage casings enable comminuted meat batters to be shaped into specific shapes as they undergo thermal processing (Osburn 2002). Collagen casings have numerous advantages over natural casings, which include 1) more uniform size, strength and flexibility required to withstand a variety of

processing environments; 2) a cleaner, more sanitary product and 3) greater consistency in net product weight (Kutas 1987). To enhance the appeal of sausage products encased in collagen casings, brown, caramel and other food grade colors are often added to provide products with distinct colors that are marketed as distinctive, smoked, or spicy specialty products. This practice is often employed because non-colored casings tend to appear gray after steam cooking (Osburn 2002). Heat cured WPI-based films provide the benefit of a natural pigmented appearance similar to caramel or smoked colored casings without the addition of food coloring. Increased yellowness (demonstrated by increased Hunter Lab b/yellowness values) has been reported following heat curing of these films (Kim and others 2002; Cagri and others 2003). These findings further indicate the potential for WPI-based films to be used as sausage casings.

Previous research from our lab has shown that heat-cured (80, 90 and 100°C for 12, 24, 48 and 72 h) WPI-based films plasticized with glycerol and containing candelilla wax have mechanical properties similar to collagen films (Amin and others 2003). A WPI-based film that can withstand meat process conditions may provide the meat industry with an alternative to collagen films. Therefore, the objective of this research was to determine if heat-cured WPI-based edible films could withstand the temperature, time and RH processing conditions encountered in a comminuted meat cooking processing scheme typically used for Polish sausage manufacture.

2.3. MATERIALS AND METHODS

2.3.1. Materials

Whey protein isolate (WPI, Provon 190) was supplied by Glanbia ingredients (Monroe, WI). Glycerol was purchased from J.T. Baker Co. (Phillipsburg, NJ). Sodium hydroxide (2N, NaOH) was purchased from Mallinckrodt Specialty Chemical Co. (Paris, KY). Candelilla wax (CW) was purchased from Strahl and Pitsch Inc. (West Babylon, NY). Edible collagen casings (inside diameter, 32mm) were obtained from The Brechteen Co. (Chesterfield, MI). Magnesium nitrate, lactose, boric acid, hydrochloric acid, methylene blue and sodium hydroxide were purchased from Sigma Chemical Co. (St Louis, MO) and preweighed Kjeldahl catalyst tablets were purchased from Fisher Scientific (Pittsburgh, PA).

2.3.2. Proximate analysis

Proximate analysis was conducted to verify the composition of the whey protein isolate (WPI). The values were compared to specifications provided by Glanbia Products.

2.3.2.1. Moisture content

Moisture content was determined according to the AOAC gravimetric method 927.05 (2000). A 1.0 – 1.5g sample of WPI was weighed into a flat-bottomed metal dish and dried in a convection oven at 130°C until equilibrium was reached. Equilibrium was reached when the sample was at constant weight and this was determined by weighing the sample at regular time intervals. Samples were removed from the oven, allowed to cool in a dessicator and weighed. Moisture content was determined as loss of sample

weight during drying and expressed as a percentage of initial weight of sample [(initial sample weight – dried weight)/(initial weight of sample)] x 100).

2.3.2.2. Protein content

Protein content was determined according to the AOAC micro Kjeldahl method 930.29 (2000). A 0.06g sample of WPI was weighed and portioned into a Kjeldahl digestion flask with ½ a catalyst tablet [(3.5g K_2SO_4, 3.5mg Se) or (3.6 x 10^{-4} M K_2SO_4, 3.6 x 10^{-4} mM Se)]. The sample was heated to digest the WPI until the solution was clear, ~6 h. Approximately 20 ml of 30% NaOH was added to the digested sample and this was distilled into a flask containing 10ml boric acid plus 2 drops methylene blue indicator. The digest was then diluted with water (5ml) and titrated with standardized 0.0501 N HCl. Percent protein was calculated using the equation: %protein = (vol. HCl) x (1.4007) x (6.38) x (normality of HCl)/sample weight in g; where 6.38 is the protein conversion factor.

2.3.2.3. Ash

Ash content was determined using the AOAC gravimetric method 930.30 (2000). A 2.0g WPI sample was weighed into a crucible and ashed in a muffle furnace at 600°C until it was carbon-free (2½ hours). The crucible was then removed from the furnace, allowed to cool in a dessicator and weighed. Ash content was determined as the (residue/initial weight of sample) x 100.

2.3.2.4. Lactose content

Lactose content was determined using high pressure liquid chromatography (HPLC). The WPI sample was prepared according to the method described in Standard Methods for the Examination of Dairy Products (Marshall 1992) with the following

changes: a 5.0g sample of WPI was dissolved in 20 ml of deionized H_2O. From this solution, a 10.0g sample was obtained to which 1 ml of 0.9N H_2SO_4 was added; water was then added to yield 100 ml and the solution was shaken for 20 s. This solution was allowed to stand for 1 h, then filtered through a Whatman #41 filter. Samples were separated on a Waters HPLC system using a Rezex RNM carbohydrate column (Phenomenex, Torrance CA). Lactose in the sample was quantified using a Waters refractive index detector (Waters, Milford MA). The areas under the sample and standard (0.5% w/v) lactose peaks were determined (in cm^2). These areas were used to determine the lactose levels. The reportable sample lactose value (%) was calculated from the equation shown below.

% recovery = [Y(a) – 4.75)/X(a)] x 100, where Y(a) = standard lactose level, X(a) = sample lactose level and 4.75 is a constant.

2.3.3. Whey protein isolate based film preparation

WPI (5% w/v) was dissolved in distilled water, glycerol (3.3% w/v) was added and pH was adjusted to 8 with 2N NaOH. The solution was heated to 90 $\pm$ 2°C while being stirred continuously to denature the whey protein. Candelilla wax (0.8%, w/v) was added during heating and allowed to melt into the solutions to provide a final solids content of 5.8% w/v for the film forming solution. The solution was homogenized for 2 minutes using a Polytron PT 10/35 homogenizer with a PTA 20 TS homogenizing head (Tekmar Co., Cincinnati, OH) at speed 5 (1350 rpm). The film forming solution was filtered through a layer of cheesecloth, equilibrated to room temperature for 1.5 h and degassed using a vacuum pump at room temperature for 30 min. Films were prepared by casting the film forming solution (27.5 ml) onto 18.5 cm circular Teflon® plates (Kim

2000). The films were dried at room temperature: 23 ± 2°C and 30 ± 5% relative humidity (RH) for 18 ± 3 h. Dried films were peeled, wrapped in foil and heat cured in a vacuum (30mm Hg) oven maintained at 1) 80°C for 24h or 2) 90°C for 12 h. The films were removed from the oven and peeled from the foil while still warm for ease of removal. The films were subsequently conditioned at 50% RH, 23°C in a dessicator containing saturated magnesium nitrate for 48 h prior to testing (Figure 2.1). Standards for the use of aqueous salt solutions for maintaining constant RH are well documented (ASTM 1997).

2.3.4. Collagen films

Collagen films were conditioned in a similar way to WPI-based films. Collagen film thickness and mechanical properties: TS and %E were evaluated for comparisons with heat cured WPI-based films. These analyses were conducted simultaneously to assure direct and meaningful comparisons.

2.3.5. Film thickness

Films were cut into strips (101.6mm x 25.4mm) using a Precision Sample Cutter (Thawing Albert Instrument Co., Philadelphia, PA). Film thickness was determined using a TMI model 549M micrometer (Testing Machines, Inc. Amityville, NY). Five different measurements were taken in random locations within the filmstrip test area and the mean values were used for calculations of mechanical properties.

2.3.6. Film treatment

The film strips were hung in sausage sticks using flexible wire and put through a cooking process typical for Polish sausage manufacture in a smokehouse; Enviro-Pak,

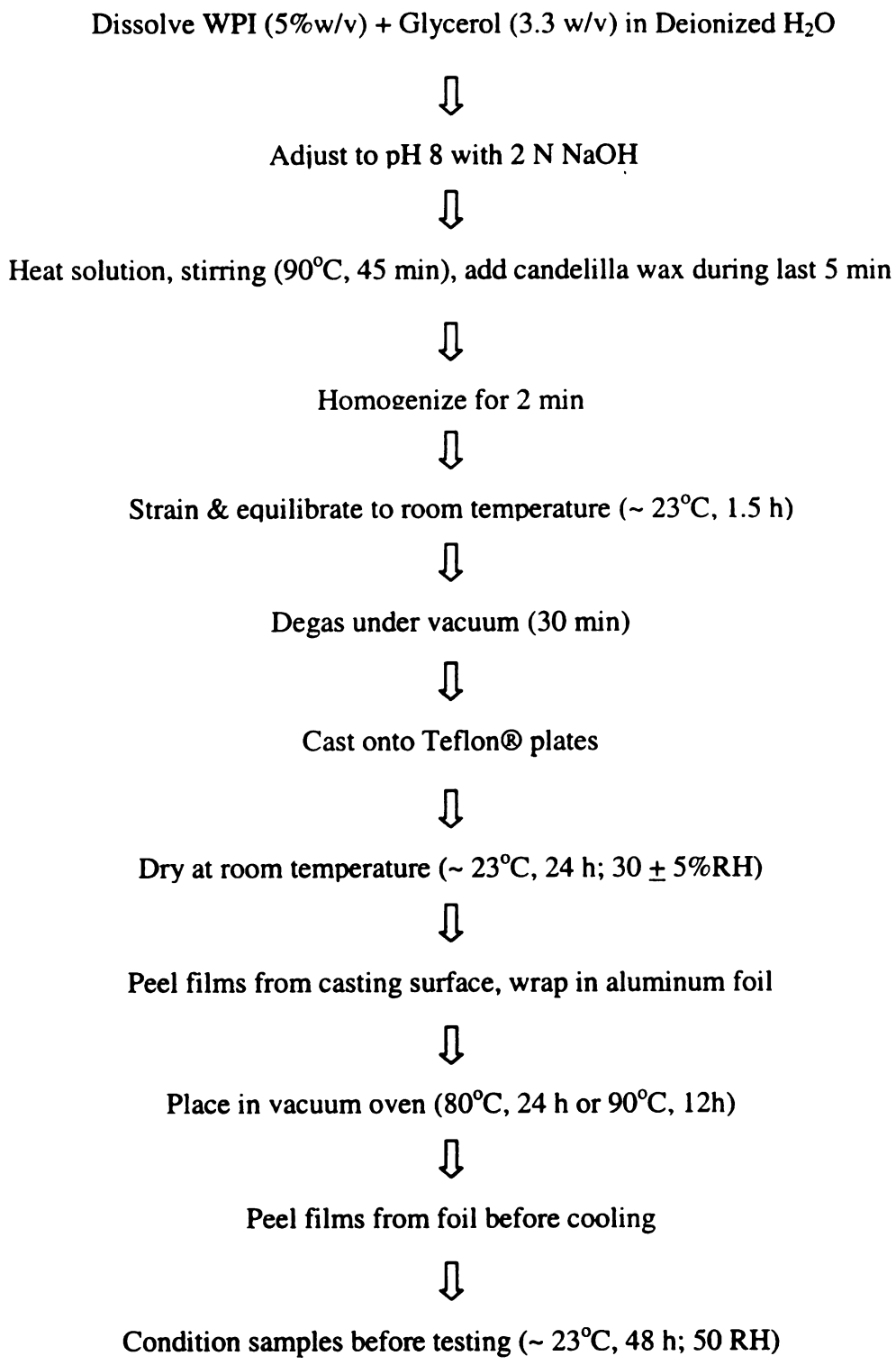


Figure 2.1 Schematic diagram of film forming procedure

WPI = whey protein isolate, RH = relative humidity

CHU-150E; Micro-Pak series MP 500 (Clackamas, OR). The meat process conditions were *stage 1*: 57°C/60 min/36%RH; this is the initial drying step in the cooking process. It is done to create a uniformly dry surface to regulate the smoke absorbed by the product; *stage 2*: 65°C/90min/60%RH; this simulates the cooking step and *stage 3*: 77°C/30min/80%RH; the finishing step where the product is cooked to target internal temperature (72°C). The intent of the high RH in stage 3 is to increase heat transfer to the center of the product. Processing began with six samples and two samples were taken for testing at the end of each stage. Samples in stage 3 underwent the entire cooking process, whereas, control samples did not undergo any processing (Figure 2.2).

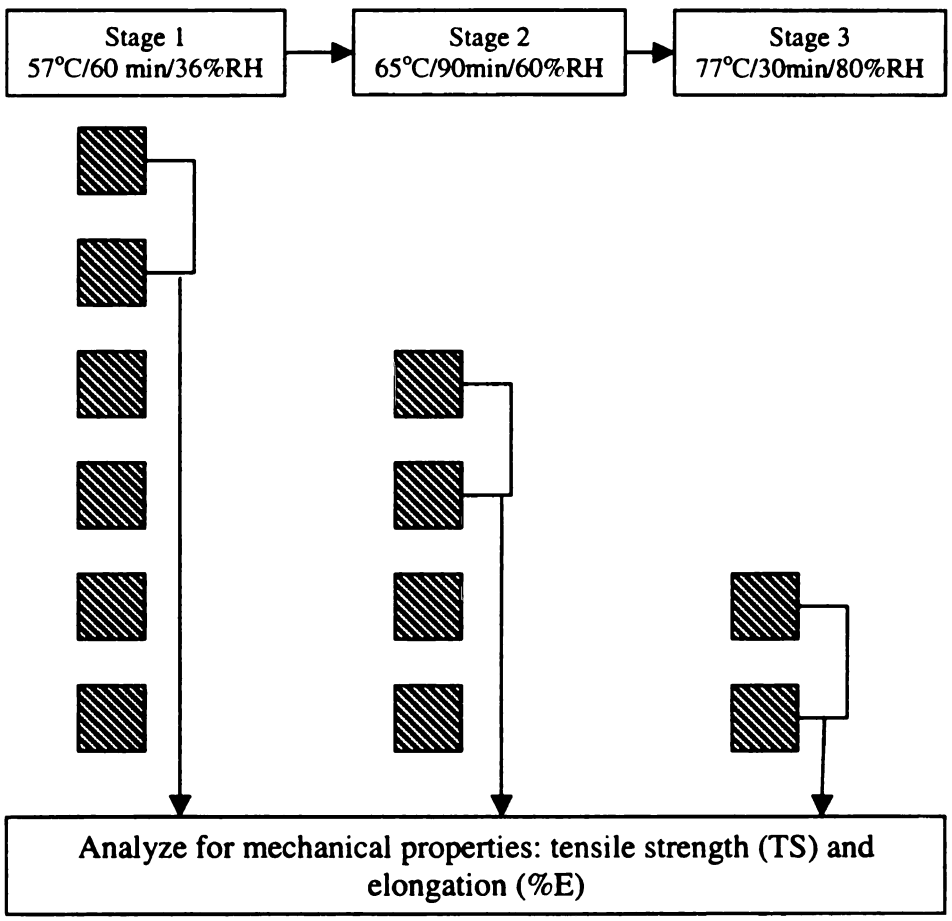


Figure 2.2 Schematic diagram of meat processing simulation, each square represents a film sample. Stages 1, 2 and 3 represent sausage cooking stages.

2.3.7. Mechanical properties

Film strips were evaluated immediately in a room maintained at 50% RH and 23°C after removal from the smokehouse. TS and %E were determined according to standard D882-91 (ASTM 1995) using the Instron Universal Testing Machine Model 2401 (Canton, MA) at 23 ± 2°C and 50 ± 5% RH. A 1kN static load cell with a crosshead speed of 50.8 cm/min was used.

2.3.8. Statistical analysis

All the experiments were replicated three times in a randomized design. A new film forming solution and new set of films was prepared for each replicate. Comparisons between means were determined using the Student-Newman-Keuls method for multiple comparisons and statistical significance was tested at ($p<0.05$). Sigma Stat (Jandel Corp., San Rafael, CA) was used to conduct statistical analysis and for mean comparisons.

2.4. RESULTS AND DISCUSSION

2.4.1. Whey protein isolate composition

Table 2.1 shows the composition of the WPI powder used for the production of WPI films. The WPI composition determined was consistent with the specifications provided by Glanbia products (Monroe, WI).

2.4.2. Film thickness

Thickness of the films was 0.14 ± 0.02 mm for films heat-cured at 80°C for 24 hours, 0.14 ± 0.02 mm for films heat-cured at 90°C for 12 hours and 0.11 ± 0.02 mm for collagen films. There was no statistical difference in thickness between any of the films.

The WPI-based film thickness values were consistent with range of film thickness previously reported by Kim (2000) which were 0.14 ± 0.02 mm. They were, however, higher than those found by Vachon and others (2000), who reported thicknesses of 0.05-0.06 ± 0.002 mm for WPI-based films cross-linked by heating and irradiation. The difference may have resulted from differences in film forming solution formulations, that is, in this study, CW was incorporated in to the films while Vachon and others (2000) did not incorporate lipids.

Table 2.1 Compositional analysis of the whey protein isolate powder utilized for whey protein isolate based films.

	Experimental Mean Values[1] (%)	Supplier's Specifications (%)
Protein	92.08 ± 0.65	92.0-95.0[2]
Lactose	0.012 ± 0.004	<1.0
Ash	2.64 ± 0.01	<3.0
Moisture	4.11 ± 0.33	<5.0

[1]n=3 for all components tested.
[2] Protein was determined on a dry basis and a nitrogen conversion factor of 6.38

2.4.3. Mechanical properties

2.4.3.1.Tensile Strength

Before the meat processing treatment, WPI-based films heat-cured at 80°C for 24 h had lower TS than collagen films ($p<0.05$). Although the TS declined during the cooking process, this decrease was not statistically significant (Figure 2.3). The initial TS of WPI-based films heat-cured at 90°C for 12 h was similar to that of collagen films; during the second cooking stage, however, the TS of the WPI-based films declined

($p<0.05$; Figure 2.4). Tensile strength of collagen films did not decrease at any time during the multi-stage cooking process

Several researchers have reported decline in TS as RH increases. Cho and Rhee (2002) reported on the effect of RH on mechanical properties of SPI-based films plasticized with glycerol (0.3-0.7 g/g SPI). These films had a TS of 14-32 MPa at 11% RH, which decreased to 1-4 MPa when the films were stored at 75% RH. They reported that at higher RH, the films absorbed moisture that resulted in decreased TS. Moisture sorption isotherm (MSI) data has also indicated the sensitivity of WPI-based films to increases in RH. Coupland and others (2000) reported that the moisture content of WPI-based films increased slowly as RH increased up to $a_w \sim 0.75$ after which it increased very rapidly.

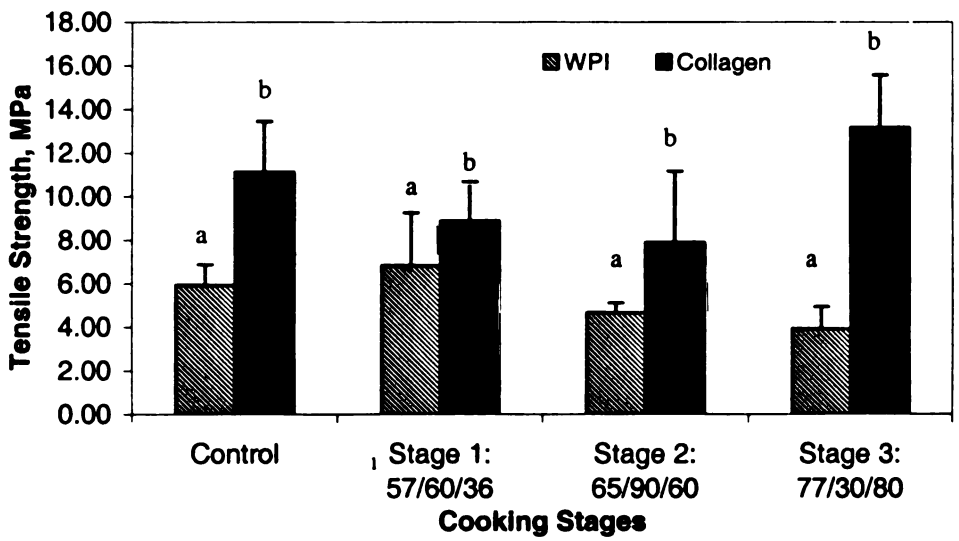


Figure 2.3 Tensile strength of whey protein isolate based films heat-cured at 80°C for 24 hours and collagen films subjected to Polish sausage manufacturing conditions. n = 3 for all treatments. WPI = whey protein isolate, control films did not undergo processing; [a-b]Different letters denote significant differences; cooking stage conditions: [1]°C/min/%RH

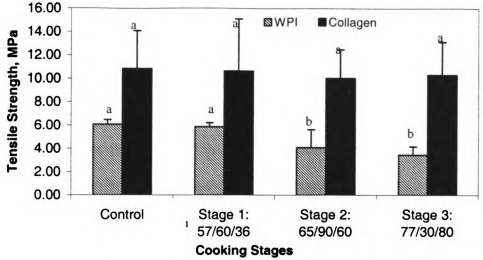


Figure 2.4 Tensile strength of whey protein isolate based films heat-cured at 90°C for 12 hours and collagen films subjected to Polish sausage manufacturing conditions. n = 3 for all treatments. WPI = whey protein isolate, control films did not undergo processing; [a-b]Different letters denote significant difference; cooking stage conditions: [1]°C/min/%RH

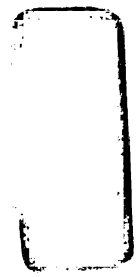
Similar findings were reported by Kim and Ustunol (2001a) for WPI-based films plasticized with glycerol or sorbitol and containing butterfat or CW. Equilibrium moisture content (EMC) of their films increased slowly up to $a_w \geq 0.65$, further increase in a_w increased EMC dramatically. Glycerol-plasticized films had higher EMC than sorbitol-plasticized films, this was attributed to the higher hygroscopicity of glycerolcompared to sorbitol. The presence of butterfat and CW reduced the EMC for both plasticizer types. Chick and Hernandez (2002) reported on the MSI of lactic-acid casein films plasticized with sorbitol and containing CW or carnauba wax. The films had low moisture uptake up to 70% RH but this rapidly increased after 85% RH. Relative humidity was shown to decrease TS of the films, at 75% RH, films were 1/5 of the strength of those maintained at 50% RH. The decline in TS observed in this study is consistent with the findings of Chick and Hernandez (2002). Based on their results, we

can conclude that it was the increase in RH during the cooking process that lowered the TS of the films.

The higher TS of films heat cured at 90°C for 12h compared to films heat-cured at 80°C for 24 h was consistent with findings by Kim and others (2002) who reported that as curing temperature increased, TS of SPI films increased. They showed that films heat-cured at 85°C had higher TS than films heat-cured at 60 or 72.5°C. Kim and others (2002) standardized heat curing of films at 24 h for all curing temperatures. Increases in TS from 8.2 to 15.8 MPa following heat-curing (90°C for 4h) was also reported by Rhim and others (2000) for SPI films. The increase in heat-curing temperature is reported to increase the formation of covalent bonds between protein chains and as water is evaporated, closer interaction occurs between the protein chains resulting in increased TS.

2.4.3.2. *Elongation*

There was no difference between the %E of WPI-based films and collagen films both before and after processing at both heat curing conditions of the films for all cooking stages (Figures 2.5 and 2.6). With the increase in RH, an increase in %E was expected as it was previously reported by Chick and Hernandez (2002). They reported that as RH increased from 50% to 75%, %E of lactic-acid-casein films containing 10% CW increased from 71 ± 26% to 116 ± 17%. Such an increase was not observed in the current study (Figures 2.5, 2.6). Kim and others (2002) reported that heat-curing SPI-based films resulted in decreased %E. Similar results were reported by Rhim and others (2000) for SPI heat cured at 90°C for 24h. Heat curing also decreases solubility of films in water. Kim and others (2002) reported that as curing temperature increased, solubility



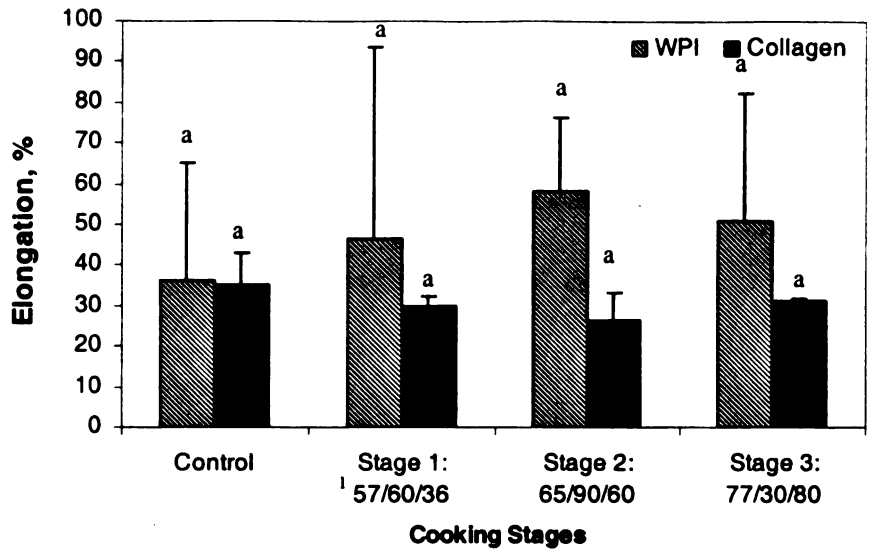


Figure 2.5 Elongation of whey protein isolate based films heat cured at 80°C for 24 hours and collagen films subjected to Polish sausage manufacturing conditions. n = 3 for all treatments, WPI = whey protein isolate, control films did not undergo processing;
[a]Same letter denotes no significant differences; cooking stage conditions: [1]°C/min/%RH

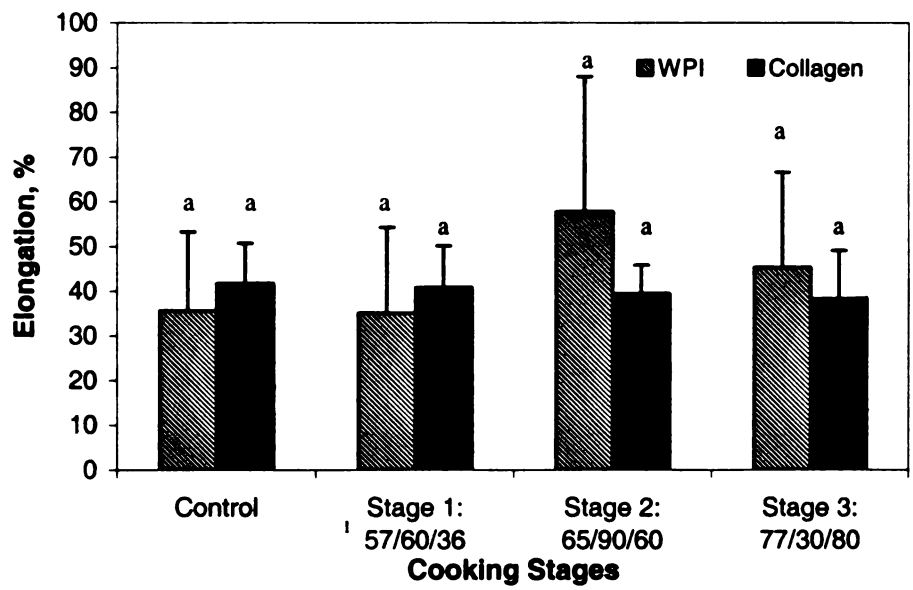


Figure 2.6 Elongation of whey protein isolate based films heat cured at 90°C for 12 hours and collagen films subjected to Polish sausage manufacturing conditions. n = 3 for all treatments, WPI = whey protein isolate, control films did not undergo processing;
[a]Same letter denotes no significant differences; cooking stage conditions: [1]°C/min/%RH

of SPI films decreased; Rhim and others (2000) reported a 12-fold decline in solubility of SPI films heat cured at 90°C for 24 h. The reduction in solubility was attributed to cross-linking resulting from the heat-curing treatment (Gennadios and others 1996; Perez-Gago and Krochta 1999). Reduced solubility also suggests reduced hydrophilicity in the films; a reduction in hydrophilicity would be expected to reduce elasticity (%E) because the plasticizing effect of adsorbed water would be lost. It is possible that no increase was seen in %E because the films were heat cured. Heat curing induces protein cross-linking, which in turn reduces water adsorption (Perez-Gago and Krochta 2001). This may explain why %E in this study did not increase with RH during processing as would be expected. Because the films were heat cured, their water adsorption was reduced; had adsorption not been reduced %E might have increased with increasing RH.

Decreases in %E resulting from heat-curing were also reported by Miller and others (1997) for WPI-based films heat cured at 60, 70 and 80°C. They suggested that the moisture content of a film determines its mechanical properties. Since moisture content is decreased during heat curing, this may result in decreased %E since water has a plasticizing effect on protein films. This, however, was not the case with the films observed in this study (Figures 2.5, 2.6). It has also been reported that effective plasticizers will generally resemble most closely the structure of the polymer they plasticize. Protein films are, therefore, best plasticized by hydroxyl compounds like glycerol and glycol (Banker 1966). Water being a hydroxyl compound would, therefore, have plasticizing effects on protein films. Lim and others (1999) reported that absorbed water and glycerol plasticize protein films synergistically resulting in films that are more flexible. The stabilization of the %E of the films in this study may also be attributed to

the added glycerol used to plasticize the films. This would suggest that glycerol was an effective plasticizer for the WPI-based films. The addition of CW to the WPI-based films may be yet another reason the %E was not affected by the cooking process. The CW added to the films may have increased their hydrophobicity making them more resistant to the effect of RH.

2.5. CONCLUSIONS

Heat curing temperature was effective in increasing TS of WPI-based films, evidenced by the similarity between films heat cured at 90°C for 12 h to collagen films. The TS of WPI-based films decreased with the increased temperature, time and RH of the cooking process. Even though the statistics showed similarity between WPI-based films and collagen films, the TS of WPI-based films was lower ($p<0.05$) than that of collagen films (6 MPa vs. 12 MPa for control films) respectively. The lower TS of WPI-based edible films demonstrated the need to improve their mechanical properties to enable their use in the high RH sausage cooking conditions. The similarity between the %E of WPI-based films and collagen films indicates a potential for WPI-based films to be used as sausage casings. The RH increase directly affected the TS of WPI-based films, however, there was sufficient hydrophobicity and plasticizer within the protein-film matrix to retain the %E properties.

CHAPTER 3

OPTIMIZATION OF MECHANICAL PROPERTIES OF WHEY PROTEIN ISOLATE BASED EDIBLE FILMS FOR USE IN SAUSAGE MANUFACTURE

3.1. ABSTRACT

Edible films were produced using WPI (5 or 5.5% w/v), glycerol (3.3 or 3.6% w/v) and candelilla wax (CW; 0.8 or 0.9% w/v) to provide film forming solutions of 5.8% w/v and 6.4% w/v solids content. Films were cast on anodized and non-anodized Teflon® surfaces. Film treatments included heat curing at 90°C for 12 h and compression molding at single-stage (110°C, 1.24 MPa, 1 h or 2 h) and double stage (110°C, 0.99 MPa, 25 min and 110°C, 1.24 MPa, 12 min) conditions. A separate compression molding experiment was conducted to optimize temperature (90, 98, 110 and 121°C), pressure (0.99, 1.10, 1.24 and 1.38 MPa) and time (2, 12 and 22 min) using response surface methodology. All films were evaluated for mechanical properties: tensile strength (TS) and elongation (%E). Film forming solution solids content and casting surface affected %E but not TS. The %E of WPI-based films cast on anodized surface after heat curing was higher than that of uncured films; %E of 5.8% w/v solids films was lower than that of 6.4% w/v solids films on non-anodized surfaces. Heat curing and compression molding had similar effects on TS and %E of uncured WPI-based films; they increased TS and did not change %E.

3.2. INTRODUCTION

Mechanical properties such as tensile strength (TS) and elongation (%E) are important properties in casings to be used in high speed automated processes like sausage manufacture. Sausage casings must be able to shrink and stretch to allow contraction and expansion of meat batter during processing. They must also be strong enough to hold the meat batter during cooking and yet tender enough to be pliable (Osburn 2002). Processing of sausage includes stuffing, linking, cooking, packaging and storing (Osburn 2002). For whey protein isolate (WPI)-based films to be used in food applications like sausage manufacture, their mechanical properties need to be optimized so that they are comparable to commercial casings currently used in the manufacture of sausage. Mechanical properties of protein edible films have been optimized using different cross-linking means including heat denaturation (Rhim and others 2000; Perez-Gago and Krochta 2001), heat curing (Rhim and others 2000; Kim and others 2002) and compression molding (Mo and others 1999; Mo and Sun 2000; Micard and others 2000). Gennadios and others (1996) reported that heat curing soy protein films at 80 or 90°C resulted in films with increased TS and reduced %E. Miller and others (1997) reported similar results with heat cured whey protein films. They suggested that heat curing elicits additional cross-linking of proteins. Rhim and others (2000) also investigated heat curing of soy protein isolate (SPI) films. In compression molding studies, Mo and others (1999) investigated the effect of molding temperature, pressure and time on TS and %E. Their findings showed that at low temperature, it took a longer time to reach maximum TS and %E. Increase in temperature resulted in decreased mechanical properties. They reported similar effects with increasing pressure. Micard and others (2000) investigated the effect

of molding temperatures without the application of pressure on wheat gluten (WG). They reported that as temperature increased, TS increased while %E decreased. Sothornvit and others (2003) have conducted a unique study on the compression molding of WPI-based films. Their results showed that compression molding was a viable method for development of WPI-based edible films.

Other means used for improving protein films mechanical properties include: the use of enzymes (Larré and others 2000), irradiation (gamma and ultraviolet) (Rhim and others 1999; Micard and others 2000; Vachon and others 2000), formaldehyde (Micard and others 2000), calcium salts (Galietta and others 1998; Fang and others 2002; Cagri and others 2002), protein concentration (Chick and Ustunol 1998; Fang and others 2002) and pH (Gennadios and others 1993a). To increase the flexibility of protein films, plasticizers are added to the film forming solution; by increasing the free volume within the protein matrix, they allow for mobility and in this way increase %E (Banker 1966; McHugh and Krochta 1994d; Sothornvit and Krochta 2001). Flexibility in WPI-based films to be used as casings is important because they need to be pliable enough for stuffing and linking. To increase the hydrophobicity of protein films, lipids or waxes such as butterfat, beeswax, carnauba and candelilla wax (CW) are added to the film forming solution. Increasing the hydrophobicity of protein films reduces their moisture uptake when placed in high relative humidity (RH) (Kim and Ustunol 2001a, Chick and Hernandez 2002). Low moisture uptake with increased RH is necessary for WPI-based films to be used in sausage manufacturing because proteins are hydrophilic and the cooking process uses elevated RH. Candelilla wax was also reported to increase TS of protein films (Kim 2000). The current study investigated ways of improving the

mechanical properties (TS and %E) of WPI-based films for the possibility of using the films as sausage casings. Heat curing and compression molding were used as physical means to improve the properties while other methods like increasing solids content and casting surface were also investigated.

3.3. MATERIALS AND METHODS

3.3.1. Materials

Whey Protein Isolate (WPI, Provon 190) was supplied by Glanbia ingredients (Monroe, WI). Glycerol was purchased from J.T. Baker Co. (Phillipsburg, NJ). Sodium hydroxide (2N, NaOH) was purchased from Mallinckrodt Specialty Chemical Co. (Paris, KY). Candelilla wax (CW) was purchased from Strahl and Pitsch Inc. (West Babylon, NY). Edible collagen casings (inside diameter, 32mm) were obtained from The Brechteen Co. (Chesterfield, MI). Magnesium nitrate was purchased from Sigma Chemical Co. (St Louis, MO) and phosphate solution was obtained from Butcher and Packer Supply Company (Detroit, MI).

3.3.2. Film formation

Figure 3.1 shows a schematic diagram of the experiments conducted under this objective designed to optimize the mechanical properties of the films. Whey protein isolate (WPI) (5 or 5.5% w/v) was dissolved in distilled water and glycerol was added (3.3 or 3.6% w/v). Then, pH was adjusted to 8.0 with 2 N NaOH. The solution was heated to 90 $\pm$ 2°C while being stirred continuously to denature the whey protein.

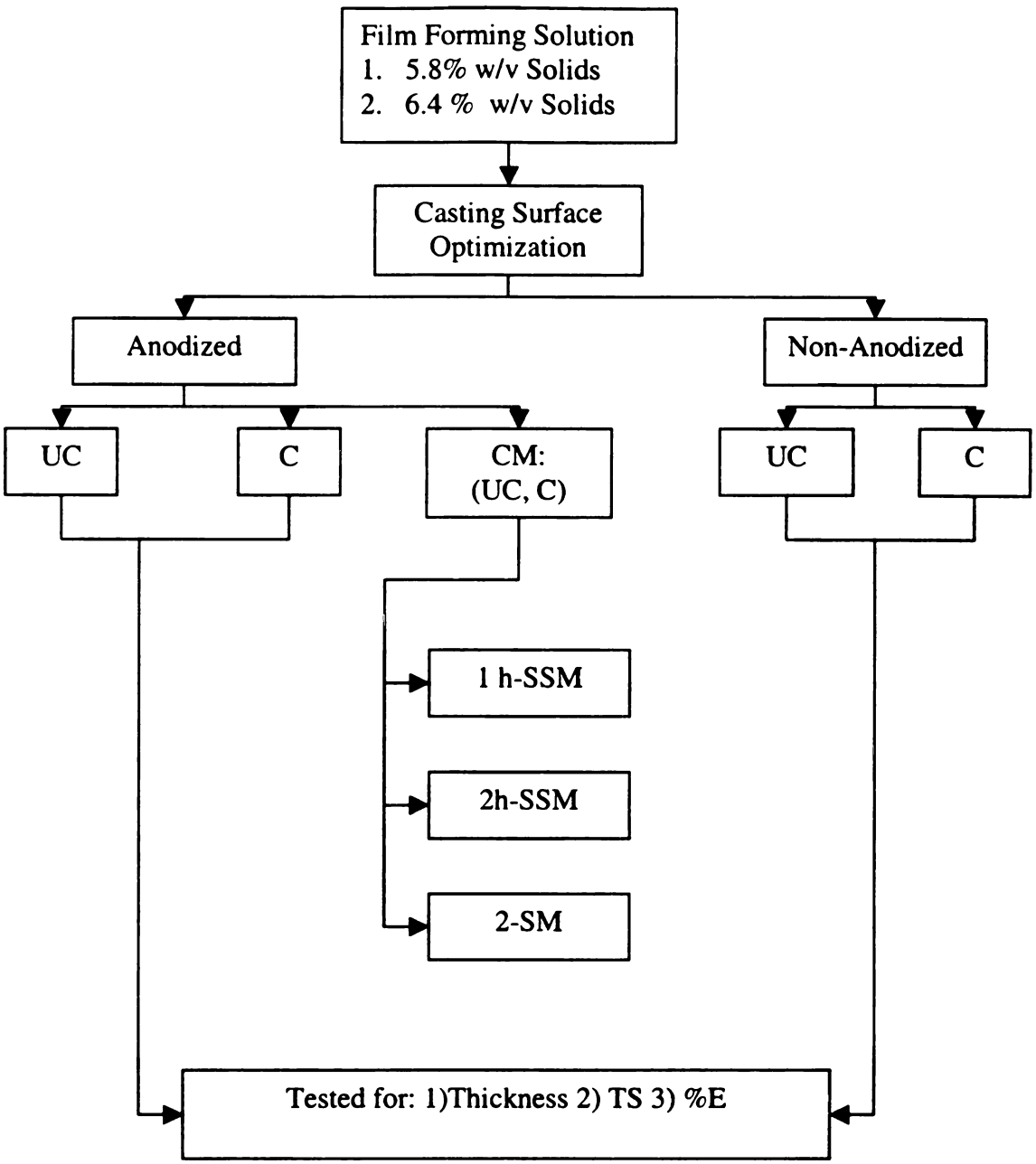


Figure 3.1. Schematic diagram of the film optimization process for whey protein isolate based films

UC = uncured, C = cured, CM = compression molded, NCM = non-compression molded, SSM = Single stage compression molded (110°C, 1.24 MPa 1 h or 2 h), 2-SM = 2-stage compression molded (90-110°C, 0.99-1.38 MPa, 2-25min), TS = Tensile Strength (MPa), % E = Elongation (%).

Candelilla wax (0.80 or 0.88 %, w/v) was added during heating and allowed to melt into the solutions to provide final solids content of 5.8% or 6.4%w/v for the film forming solution. The solutions were homogenized for 2 minutes using a Polytron PT 10/35 homogenizer with a PTA 20 TS homogenizing head (Tekmar Co., Cincinnati, OH) at speed 5 (1350 rpm). The homogenized film forming solution was filtered through a layer of cheesecloth, equilibrated to room temperature for 1.5 h and degassed using a vacuum pump at room temperature for 30 min. Films were cast by pouring film forming solution (172.6 ml) onto anodized (aluminum) Teflon® or non-anodized (aluminum) Teflon® plates (33.0 cm x 45.7 cm). The process of anodizing turns the aluminum into aluminum oxide making it heavier and corrosion resistant. The films were dried at 23 $\pm$ 2°C and 30 $\pm$ 5% relative humidity (RH) for 18 $\pm$ 3 h. Dried films were peeled, wrapped in foil and heat cured in a vacuum (30mm Hg) oven maintained at 90°C for 12 h. The films were removed from the oven and peeled from the foil while still warm for ease of removal. The films were subsequently conditioned at 50% RH, 23°C in a dessicator containing saturated magnesium nitrate for 48 h prior to testing.

3.3.3. Collagen films

Collagen films were conditioned in a similar way to WPI-based films. Collagen film thickness and mechanical properties: TS and %E were evaluated for comparisons with heat cured WPI-based films. These analyses were conducted simultaneously to assure direct and meaningful comparisons.

3.3.4. Film thickness

Films were preconditioned at 23°C, 50% RH for a minimum of 8 h to enable ease of handling and cutting cut into strips (101.6mm x 25.4mm) using a Precision Sample

Cutter (Thawing Albert Instrument Co., Philadelphia, PA). Film thickness was determined using a TMI model 549M micrometer (Testing Machines, Inc. Amityville, NY). Five different measurements were taken in random locations within the filmstrip test area and the mean values were used for calculations of mechanical properties. Thickness measurements ranged from 0.11-0.16 ± 0.02 mm.

3.3.5. Compression molding

The films were compression molded using a Carver Laboratory Press (Fred Carver, Inc.; Wabash IN). Film sheets (130 x 200 mm) were sandwiched between Teflon® sheets and placed between 2 stainless steel molding plates (220mm x 220mm). Single and double stage molding conditions were: 1) single-stage (110°C, 1.24 MPa, 1 h or 2 h) and 2) two-stage (110°C, 0.99 MPa, 25 min and 110°C, 1.24 MPa, 12 min). In a separate experiment, response surface methodology was used to determine the best conditions for two-stage compression molding of heat cured films. Stage one conditions were kept constant (110°C, 0.99MPa, 25 minutes) while stage 2 conditions were altered as follows: temperature: 90, 98, 110, 121°C; pressure: 0.99, 1.10, 1.24, 1.38 MPa and time: 2, 12, 22 min (Table 3.1). Temperatures were controlled using a temperature dial that showed temperatures of the molding plates.

3.3.6. Mechanical properties

Film strips (101.6mm x 25.4mm) conditioned at 50% RH, 23°C for 48 h prior to testing were used to determine mechanical properties. Tensile strength (TS, force at failure/original cross-sectional area) and percent elongation at break (%E, increase in length/initial length) were determined according to standard D882-91 (ASTM 1995) using the Instron Universal Testing Machine Model 2401 (Canton, MA) at 23 ± 2°C and

50% RH. A 1kN static load cell with a crosshead speed of 50.8 cm/min was used. Thickness measurements were used to calculate TS.

Table 3.1. Surface response methodology design for optimization of two-stage compression molding conditions for heat cured whey protein isolate based films.

Day	Temperature (°C)	Time (minutes)	Pressure (MPa)
1	98 ± 5	2	1.10
	98 ± 5	22	1.38
	121 ± 5	2	1.38
	121 ± 5	22	1.10
	110 ± 5	12	1.24
	110 ± 5	12	1.24
2	98 ± 5	2	1.38
	98 ± 5	22	1.10
	121 ± 5	2	1.10
	121 ± 5	22	1.38
	110 ± 5	12	1.24
	110 ± 5	12	1.24
3	90 ± 5	12	1.24
	130 ± 5	12	1.24
	110 ± 5	2	1.24
	110 ± 5	22	1.24
	110 ± 5	12	0.99
	110 ± 5	12	1.38
	110 ± 5	12	1.24
	110 ± 5	12	1.24

3.3.7. Statistical analysis

The effect of heat curing (curing), casting surface (surface), total solids content of casting solution (solution) and compression molding (molding) on TS and %E of WPI-based films were determined using the models shown below:

Y = function (curing; surface; solution) (1)

Y= function (curing; compression molding) (2)

Where Y = TS or %E, curing = uncured or heat cured, surface = anodized or non-anodized, solution = 5.8 or 6.4% w/v solids and compression molding = single or 2-stage as described in section (3.3.4). All comparisons were also made to collagen films. The mixed procedure of SAS (SAS 1996) was used to analyze the data. Treatment means were compared using the least squares means (LSM) and the means were based on 3 replicates. Response surface methodology (SAS 1996) was employed to optimize the compression molding conditions. The effects of temperature, pressure and time on TS and %E were evaluated using the conditions shown in Table 3.1. A second degree polynomial model was used for fitting TS and %E values:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + e \quad (3)$$

Where Y = TS or %E; β_0, β_1, β_2, β_3, β_{11}, β_{22}, β_{33}, β_{12}, β_{13}, β_{23} = regression constants; X_1, X_2, X_3 are the coded independent variables for temperature, pressure and time and e = the error term. This experiment was repeated over three days; statistical analysis was done using the RSREG procedure of SAS (SAS 1996). Statistical significance was tested at $p<0.05$.

3.4. RESULTS AND DISCUSSION

3.4.1. Mechanical properties

3.4.1.1. Effect of heat curing, solids content of the casting solution and casting surface

Tensile strength is the maximum tensile stress a material can sustain while %E is the strain under elastic behavior at which the sample breaks (Hernandez 1997). Initially the films were cast on round non-anodized Teflon® plates, to make larger films required

to make adequate sausage casings, the films were cast on similar plates with a larger surface area. These, however, were not successful as the solution coalesced to one side. This was possibly due to the shiny finish on the non-anodized surface compared to the rougher finish on the anodized surface.

Data in Tables 3.2 and 3.3 show the significance levels of the main effects (surface, solution and heat curing) on TS and %E. Statistical analysis of these effects showed that the casting surface and solids content of the film forming solution were not significant with respect to TS; heat curing, however, was significant ($p<0.05$).

Table 3.2 Significance level of main effects and their interactions for tensile strength

Effect	Num[1] DF	Den[2] DF	F-value	Significance
Solution	1	16	0.53	ns
Surface	1	16	1.02	ns
Curing	1	16	108.01	$p<0.05$
Solution x Surface	1	16	0.59	ns
Solution x Curing	1	16	1.92	ns
Surface x Curing	1	16	4.31	$p<0.05$
Surface x Solution x Curing	1	16	6.46	$p<0.05$

[1] Num DF = numerator degrees of freedom,
[2]Den DF = denominator degrees of freedom
ns = not significant

Table 3.3 Significance levels of main effects and their interactions for elongation

Effect	Num[1] DF	Den[2] DF	F-value	Significance
Solution	1	16	1.81	ns
Surface	1	16	0.27	ns
Curing	1	16	0.01	ns
Solution x Surface	1	16	7.28	$p<0.05$
Solution x Curing	1	16	0.04	ns
Surface x Curing	1	16	5.25	$p<0.05$
Surface x Solution x Curing	1	16	0.24	ns

[1] Num DF = numerator degrees of freedom,
[2]Den DF = denominator degrees of freedom
ns = not significant

The two-way and three-way interactions: (surface x curing) and (surface x solution x curing) were significant ($p<0.05$). With respect to %E, only the interaction effects (solution x surface) and (surface x curing) were significant ($p<0.05$). Even though the three-way interaction between surface, solution and curing appeared significant, there were no differences between mean TS values among uncured or heat cured films from different casting solutions and surfaces. Two-way interactions are discussed under the TS and %E discussions. Table 3.4 shows the mechanical properties (TS and %E) of uncured and heat cured WPI-based films cast on non-anodized and anodized Teflon® surfaces from solutions with two levels of solids content. Heat cured films had higher TS than uncured films; for example, 2.00 MPa vs. 8.57 MPa for 5.6% w/v solids film ($p<0.05$). This trend was consistent with the remainder of the data. These results are in agreement with those reported by Rhim and others (2000) for SPI films. Soy protein isolate (SPI) films were prepared from heated aqueous SPI solutions (70°C for 20 min) and heat cured at 90°C for 24 h. Increases from 8.2 to 14.7 MPa were reported for uncured vs. cured films. Gennadios and others (1996) had previously showed that heat curing of SPI films at 80 or 95°C for 2, 6, 14 and 24 h increased TS and this increase was positively correlated to increase in heat curing time and temperature. These data provide adequate basis for heat curing of WPI-based films for the purpose of improving their mechanical properties. WPI-based films in this study were heat-cured at 90°C for 12 h; the increase in TS resulting from heat curing was, however, not sufficient to make the films comparable to collagen films. Tensile strength of all WPI-based films was lower ($p<0.05$) than that of collagen films (5.13-8.57 MPa vs. 13.29 MPa).

Table 3.4. Mechanical properties of uncured and cured whey protein isolate-based films cast on non-anodized and anodized surface, from solutions varying in solids content

Casting Surface	Film Solution	TS (MPa)		%E	
		Uncured	Cured	Uncured	Cured
Non-Anodized	5.8% w/v Solids	2.00 ± 0.16[a]	8.57 ± 1.12[b]	35.81 ± 9.37[a]	30.16 ± 9.25[a]
	6.4% w/v Solids[1]	2.40 ± 0.21[a]	5.13 ± 0.57[b]	42.73 ± 1.50[b]	37.64 ± 15.2[b]
Anodized	5.8% w/v Solids	2.43 ± 0.34[a]	5.85 ± 0.44[b]	43.81± 21.83[b]	56.64 ± 16.33[b]
	6.4% w/v Solids[2]	2.89 ± 0.58[a]	7.11 ± 0.24[b]	36.30 ± 10.66[a]	54.09 ± 19.10[b]
Collagen		13.29 ± 3.47[c]		31.78 ± 4.88[ab]	

[a-b]Means with the same superscript are not significantly different from each other ($p<0.05$). Comparisons are made within the same column and row for each mechanical property, n=3 for all treatments. TS = Tensile strength, %E = Elongation (%)

These results indicate that WPI-based films did not have the kind of strength required to withstand sausage manufacturing conditions. Preliminary experiments to test the performance of WPI-based films in sausage manufacturing conditions are shown in Appendix II.

Increased solids content (6.4% w/v) and non-anodized surface resulted in films with higher %E compared to 5.8% w/v solids films ($p<0.05$). Films cast on anodized surfaces had higher %E than their counterparts formed on non-anodized surfaces ($p<0.05$). The two-way interaction between casting surface and heat curing is clearly seen in the higher %E for heat cured film on the non-anodized surface, for example

30.16% vs. 56.64% for 5.8% w/v solids films cast on non-anodized and anodized surfaces respectively. Similar results were seen for 6.4% w/v solids films. The higher %E values observed for films cast on the anodized the surface suggest that casting surfaces affect mechanical properties of films cast on them. To my knowledge, no previous research has been conducted to determine the effect of casting surface on protein film properties. Various casting surfaces have been used by different researchers, however, no explanations for the use of one surface over another have been provided. Some surfaces that have been used include: Teflon®-coated glass plates (Gennadios and others 1993a, b; Cho and Rhee 2002), polystyrene Petri dishes (Choi and Han 2001) and ultra high molecular weight, high density polyethylene (Fairley and others 1996a, b). The main difference between the surfaces used in this study was that the anodized Teflon® surface was heavier and allowed better spreading of the film-forming solution. This may have contributed to a conformation that allowed better mobility of the molecules within the protein film than the non-anodized surface since the film-forming solution tended to coalesce more on the non-anodized surface. Further research is required to determine the cause of this effect.

The %E values obtained in this study are higher than those reported by Kim (2000) for similar uncured films plasticized with sorbitol. Kim studied properties of WPI-based films plasticized with glycerol and sorbitol. Mechanical properties were, however, reported only for sorbitol-plasticized films. This researcher reported %E of 15.6 ± 3.4% for these films while in this study a %E of 35.81 ± 9.37% was obtained. The %E of films in this study did not change following heat curing which is contrary to findings of other researchers who have reported that heat curing increased TS but

decreased %E (Cuq and others 2000; Rhim and others 2000; Micard and others 2000). In the study conducted by Rhim and others (2000), film strips were heat cured at 90°C for 24 h in an air-circulating oven. Heat curing decreased %E from 30 ± 3.3% for uncured films to 6.1 ± 0.7% for heat cured films.

The purpose of increasing solids content in the film forming solution was to induce changes to optimize the mechanical properties of the films. Mechanical properties are largely dependent on the structure of the film. According to Anker and others (2001) the microstructure of WPI-based films is dependent on protein concentration. Increased protein concentration results in a more aggregated structure that has a denser protein network. Chick and Ustunol (1998) studied the effect of increasing protein:plasticizer ratios (0.6:1, 1:1, 1.4:1) on lactic-acid-casein and rennet-casein-based edible films. Plasticizers studied were glycerol and sorbitol. For lactic-acid-casein films, increase in protein concentration decreased %E; for rennet casein films, however, %E increased as protein concentration increased. The results obtained for rennet-casein films are consistent with the results seen for films cast on non-anodized surfaces in this study. The difference between the two studies is that total solids in this study were increased while maintaining the protein:plasticizer ratio, whereas, protein:plasticizer ratio was altered in the study by Chick and Ustunol (1998). The results presented by Chick and Ustunol (1998) indicate that a higher increase in the protein concentration in the current study may have yielded more significant effects on the mechanical properties of the WPI-based films.

3.4.1.2. Compression molding

Figure 3.2 shows TS of compression molded films from the initial compression molding experiments. Compression molding resulted in increased TS of uncured films

($p<0.05$). This increase was similar to that caused by heat curing, this is shown by the heat cured unmolded film on the same figure (Figure 3.2). Single stage compression molding conditions yielded similar results and there was no difference between uncured and heat cured films compression molded under these conditions.

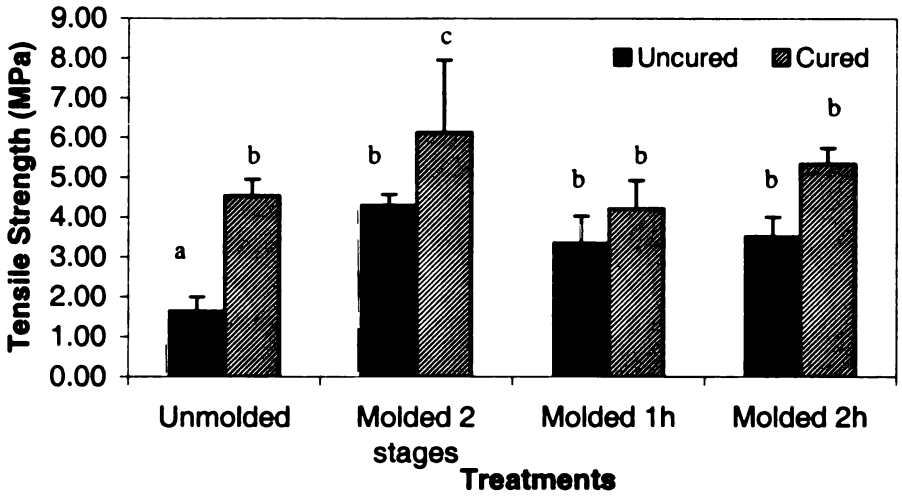


Figure 3.2. Tensile Strength of compression molded whey protein isolate based films.
[a-c]Different letters denote significant differences; n = 3 for all treatments;
Molded 2 stages = 2 stage molding: 110°C, 0.99 MPa, 25 minutes and 110°C, 1.24 MPa, 12 minutes, Molded 1h or 2h = single stage molding: 110°C, 1.24 MPa, 1 or 2 hours

Two-stage compression molding significantly increased ($p<0.05$) the TS of heat cured films compared to uncured films. Sothornvit and others (2003) reported that a feasible temperature range for compression molding of WPI-based films was 104°C – 140°C. Adequate film formation without degradation was obtained at temperatures lower than 140°C. The pressures they studied ranged from 0.81MPa – 2.25 MPa. Compression molding was conducted for 2 minutes because it was observed that at high temperature (140°C), films degraded after 2 minutes. Film degradation was evidenced by the darkening of the film color. The film testing conditions used in this study were similar to

the conditions used by Sothornvit and others (2003). These researchers, however, did not report mechanical properties and thus it was not possible to compare the effect of compression molding on TS and %E of WPI-based films. The increased TS resulting from heat application to free-standing films is consistent with results reported by Micard and others (2000) for WG films. They heat treated WG films by placing them between two plates heated at 80, 95, 110 and 125°C for 15 min and at 140°C for 1.5 or 15 min without the application of pressure. The TS increased from 1.7 MPa for non-treated films to 3.1 MPa at 110°C and 7.3 MPa at 140°C. A two-fold increase in TS was reported as temperature increased from 110°C to 125°C. A similar trend was seen in this study with the WPI-based films since uncured, un-compression molded films' TS increased from 1.65 MPa to 4.31 MPa after 2-stage compression molding at 110°C (Figure 3.2).

The larger increase in the TS of the WPI-based films could be due to the longer compression time, 25 and 12 min compared to 15 min used by Micard and others (2000) as well as the application of pressure, which they did not use. The results obtained by Micard and others (2000) suggest that increased TS for WPI-based films may be merely due to the increase in temperature since these results are consistent with the results reported for heat curing of protein films and heat cured films in this study. Gennadios and others (1996) showed that increased heat curing temperature and time caused increases in TS of SPI films. Similar results were reported by Kim and others (2002) for SPI films heat cured at 60-85°C under 61.32-101.3 KPa. The TS of the SPI films was reported to increase as curing temperature and pressure increased. Mo and others (1999) studied the effect of molding temperature (100, 120, 140, 150, 160°C), pressure (5, 10, 20, 40, 60 MPa) and time (3, 5, 10, 15 min) on compression molded SPI polymers, they

reported that as pressure increased from 5 to 10 MPa, TS increased to 42.2 MPa but as pressure was increased to 20 MPa, TS decreased to 39 MPa and remained constant as pressure continued to increase.

Results of this current study were consistent with those of Mo and others (1999) for 2-stage compression molded heat cured films. Increasing pressure from 0.99 to 1.24 MPa increased TS. Cuq and others (2000) studied the effect of molding temperature (80, 100, 110, 125, 135, and 150°C) on WG films, pressure and time were held constant at 20 MPa and 10 min respectively. As with Mo and others (1999), they showed that increases in temperature caused increase in TS (0.26 to 2.04 MPa) as temperature increased from 80 to 135°C. The similarity in the effect of compression molding and heat curing suggests that compression molding can be used as an alternative to heat curing under vacuum. Heat curing free-standing films (Rhim and others 2000; Kim and others 2002) increases covalent cross-linking between protein polymer chains that increases water insolubility and increases tensile properties.

Compression molding had no significant effect on %E for all compression molding conditions that were investigated (Figure 3.3). The differences in %E between uncured and cured films were also not significant. These results are contrary to the findings of Mo and others (1999) and Cuq and others (2000). Mo and others (1999) reported an increase in %E that increased from 0.9 to 5.4% as pressure increased from 5 to 10 MPa and remained constant at 4.5% regardless of increase in pressure. The effect of time and temperature was studied at constant pressure (20 MPa). Mo and others (1999) found that at high temperature (150°C) it took 3 min to reach optimum curing quality (max TS and %E) and up to 10 min at low temperature (120°C). Cuq and others

(2000) reported that as molding temperature increased from 80 to 135°C, %E decreased from 468 to 236%. The difference between the films in this study compared to films studied by the researchers mentioned above is the presence of CW, which may have

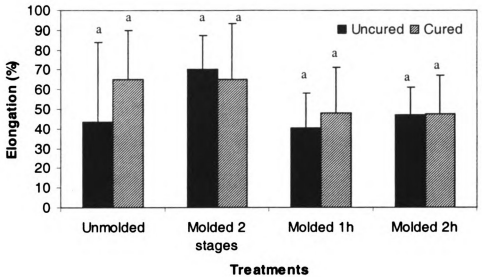


Figure 3.3. Elongation of compression molded whey protein isolate-based films. [a]Same letter denotes no significant differences between means; n = 3 for all treatments, Molded 2 stages = 2 stage molded: 110°C, 0.99 MPa, 25 minutes and 110°C, 1.24 MPa, 12 minutes, Molded 1h or 2h = single stage molded: 110°C, 1.24 MPa, 1 hour or 2 hours

allowed mobility within the polymer matrix by reducing protein-protein interactions. Callegarin and others (1997) reported that the presence of lipids might increase flexibility of films by weakening the intermolecular forces between adjacent polymer chains.

Figures 3.4-3.7 show mechanical properties (TS and %E) of WPI-based films compression molded in two stages (stage 1: 110°C, 0.99 MPa, 25 min and stage 2: 90, 98, 110,121°C; 0.99, 1.10, 1.24; 1.38 MPa, 2, 12, 22 min) as determined by response surface methodology. The effects of temperature, pressure and time on TS and %E can be seen on the response surface plots generated by equations (4) and (5) (Figures 3.4-3.7). The equations show the values of the regression coefficients.

$$TS = 26.63 - 0.22\,X_1 - 0.13\,X_2 + 0.04\,X_3 + 0.006\,X_1^2 + 0.0002\,X_2^2 + 0.0005\,X_3^2 + 0.0006\,X_{12} + 0.0002\,X_{13} - 0.0002\,X_{23} \quad (4)$$

$$\%E = 10.45 - 0.22\,X_1 + 0.06\,X_2 - 0.10\,X_3 + 0.008\,X_1^2 - 0.0002\,X_2^2 + 0.0004\,X_3^2 + 0.0002\,X_{12} + 0.0002\,X_{13} - 0.002\,X_{23} \quad (5)$$

Figure 3.4 shows the effect of temperature and pressure on TS and Figure 3.5 show the effect of temperature and time on TS. The effect of temperature and pressure on %E is shown in Figure 3.6 and Figure 3.7 shows the effect of temperature and time on %E.

Statistical analysis of the data indicated that the regression coefficients for both TS and %E were not statistically significant. Statistical insignificance of the regression coefficients suggests that increases or decreases in the variables (temperature, pressure and time) did not result in significant changes in the dependent variables (TS and %E). As a result, no combination of temperature, pressure and time was found to be optimal or to yield significant increases or decrease in TS or %E under the conditions investigated. The relatively flat nature of the response surface graphs demonstrates these results. Since the regression coefficients were not statistically significant, a reduced linear model without the quadratic and interaction terms was used to fit the TS and %E. Using the reduced model, the regression coefficient corresponding to temperature (the X_1 term) was statistically significant ($p<0.05$) and positive, indicating that an increase in temperature resulted in an increase in TS; pressure and time were not significant. Equation (6) shows the TS model. For %E, the regression coefficients of temperature, pressure and time remained statistically insignificant. This indicates that changes in these variables did not increase or decrease %E of WPI-based films. Equation (7) shows the %E model. As previously discussed, these findings were contrary to those obtained by other researchers

who have investigated similar processes (Mo and Sun 1999; Cuq and others 2000; Micard and others 2001).

$$TS = 3.19 + 0.02\,X_1 - 0.01\,X_2 + 0.01\,X_3 \quad (6)$$

$$\%E = 18.65 - 0.96\,X_1 - 0.02\,X_2 + 0.31\,X_3 \quad (7)$$

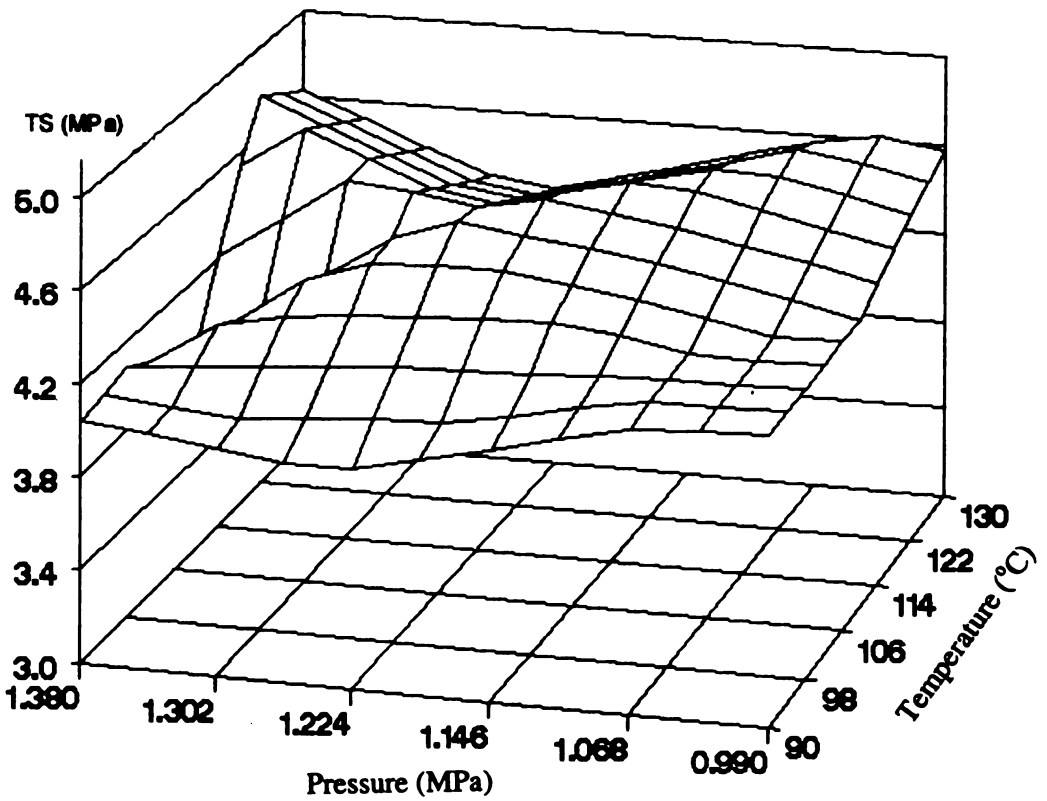


Figure 3.4. Effect of temperature and pressure on tensile strength (TS) of 2-stage compression molded whey protein isolate-based films as determined by response surface methodology.
Compression molding conditions: Stage 1: 110°C, 0.99 MPa, 25 min; Stage 2: 90, 98, 110, 121°C; 0.99, 1.10, 1.24, 1.38 MPa; 2, 12, 22 min
n = 3 for all treatments; No significant differences were found between treatment means.

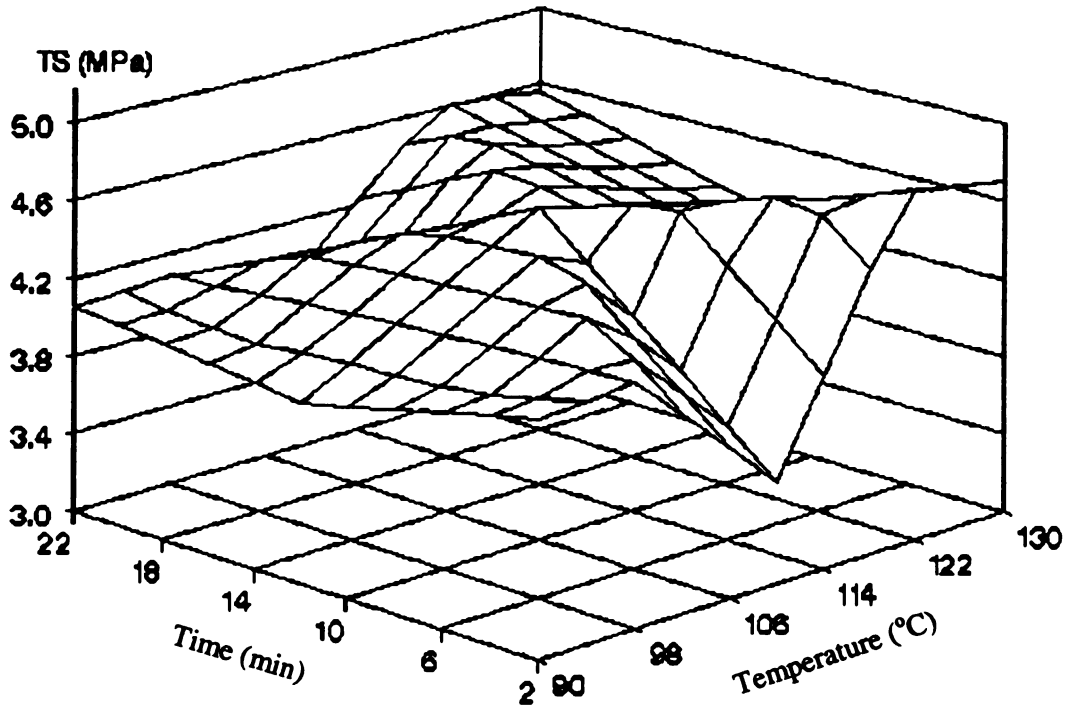


Figure 3.5. Effect of temperature and time on tensile strength (TS) of 2-stage compression molded whey protein isolate-based films as determined by response surface methodology.
Compression molding conditions: Stage 1: 110°C, 0.99 MPa, 25 min; Stage 2: 90, 98, 110, 121°C; 0.99, 1.10, 1.24 1.38 MPa; 2, 12, 22 min.
n = 3 for all treatments; No significant differences were found between treatment means.

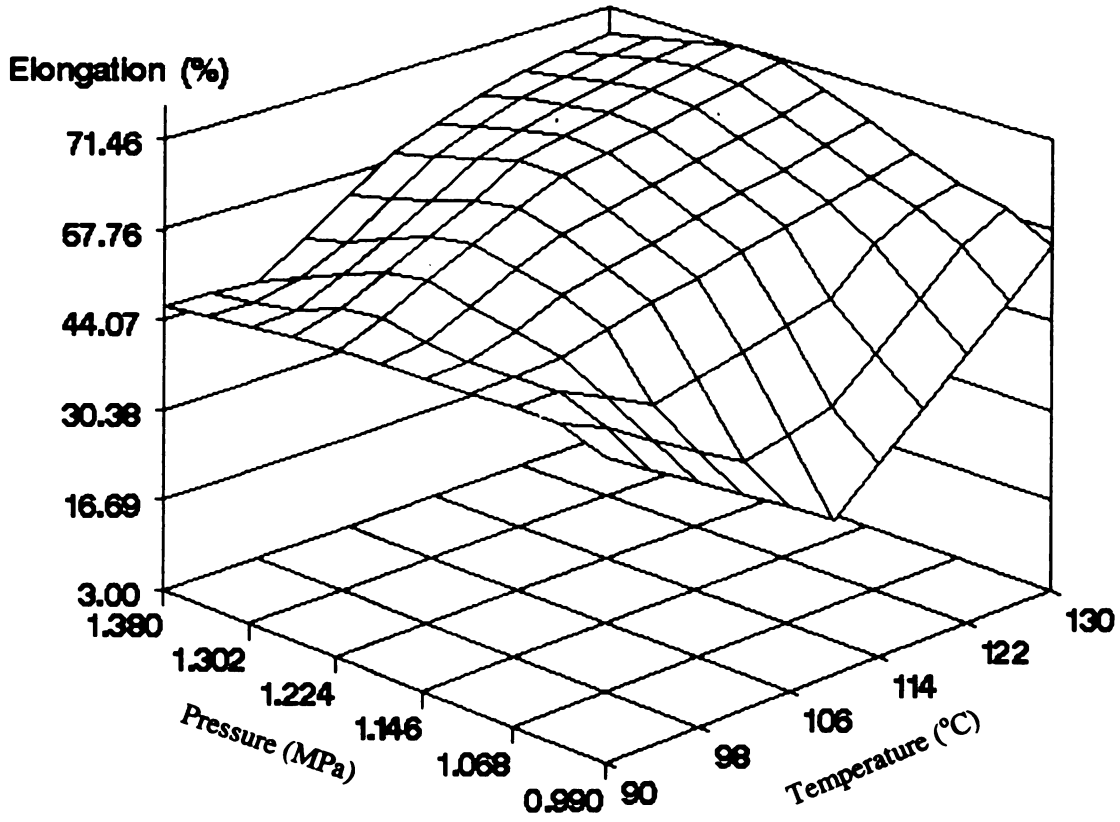


Figure 3.6. Effect of temperature and pressure on elongation (%) of 2-stage compression molded whey protein isolate-based films.
Compression molding conditions: Stage 1: 110°C, 0.99 MPa, 25 min; Stage 2: 90, 98, 110, 121°C; 0.99, 1.10, 1.24, 1.38 MPa; 2, 12, 22 min.
n = 3 for all treatments; No significant differences were found between treatment means.

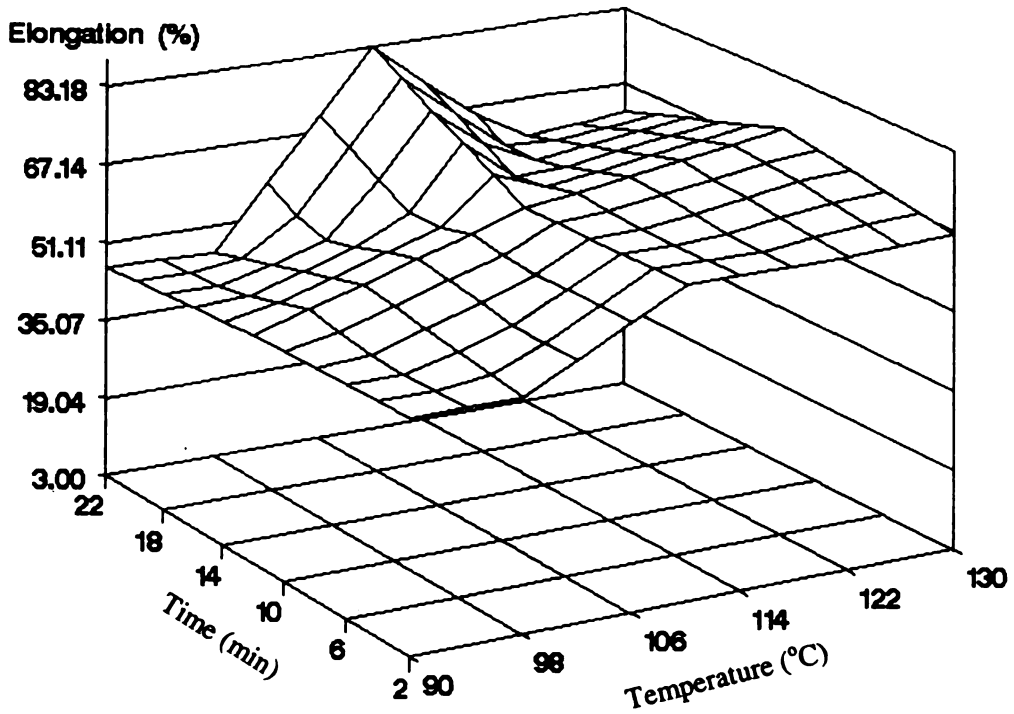


Figure 3.7. Effect of temperature and time on elongation (%) of 2-stage compression molded whey protein isolate-based films.
Compression molding conditions: Stage 1: 110°C, 0.99 MPa, 25 min; Stage 2: 90, 98, 110, 121°C; 0.99, 1.10, 1.24, 1.38 MPa; 2, 12, 22 min.
n = 3 for all treatments, No significant differences were found between treatment means.

3.5. CONCLUSIONS

Casting surface and film forming solution solids content were significant factors affecting %E but not TS of WPI-based films. Heat curing and compression molding had similar effects on TS and %E of uncured WPI-based films, they increased TS and did not change %E. Compression molding can therefore be used as an alternative to heat curing. This would be advantageous because compression molding takes less time (> 1 h vs. 12 h for heat curing), has less equipment requirements (hydraulic press vs. vacuum pump and oven) and less total energy requirements and thus more economic. WPI-based edible films had lower TS than collagen films. The lower TS of WPI-based films compared to collagen films indicated that even though the TS was increased with heat curing and

compression molding, the increase was not sufficient to enable use of these films as sausage casings. An additional experiment to optimize mechanical properties WPI-based films was conducted using potassium phosphate; this experiment is shown in Appendix I.

CHAPTER 4

OPTIMIZATION OF SEAL PROPERTIES OF WHEY PROTEIN ISOLATE EDIBLE FILMS.

4.1. ABSTRACT

Edible films were produced using WPI (5 or 5.5% w/v), glycerol (3.3 or 3.6% w/v) and candelilla wax (CW; 0.8 or 0.9% w/v) to provide film forming solutions of 5.8% w/v and 6.4% w/v solids content. Films were cast on anodized and non-anodized Teflon® surfaces. Film treatments included heat curing at 90°C for 12 h and compression molding at single-stage (110°C, 1.24 MPa, 1 h or 2 h) and double stage (110°C, 0.99 MPa, 25 min and 110°C, 1.24 MPa, 12 min) conditions. The effect of heat-sealing prior to heat curing and wetting the seal area of uncured and heat cured films before heat sealing were investigated. All films were evaluated for seal strengths. Electron Spectroscopy for Chemical Analysis (ESCA) was used to determine surface chemistry of the unsealed and sealed films. Uncured films had higher ($p<0.05$) seal strengths than cured films. Seal strengths of uncured and heat cured compression molded films were similar. Neither heat-sealing prior to heat curing nor wetting the seal area of uncured and heat cured films before sealing improved seal strengths. ESCA indicated that cured, compression molded and sealed films had higher concentrations of C-O-H and C-O-C bonds on their surface and that sealing of the films was likely a result of the formation of C-O-H or C-O-C bonds.

4.2. INTRODUCTION

Heat sealing is very widely used in the packaging industry for joining polymers (Meka and Stehling 1994). Adequate sealing is obtained when two polymer surfaces are pressed together with sufficient pressure and time to cause the polymer chains to diffuse across the interface and form bridges (Mueller and others 1998). Meka and Stehling (1994) showed that the dwell time had a relatively small influence on seal strength compared to temperature and pressure. Mueller and others (1998), however, stated that factors affecting seal strength were temperature and dwell time.

Sealability of edible films is an important attribute in the development and applications of edible films. Their sealability determines the possibility of using the films in such applications as pouches and sachets for soups and other products (Amin and others 2001; Kim 2000). Heat sealability of whey protein isolate (WPI)-based films plasticized with glycerol or sorbitol and containing candelilla wax was reported by Kim and Ustunol (2001c). They formed seals using a thermal heat sealer and reported that the optimum sealing temperatures for glycerol- and sorbitol-plasticized films were 110°C and 130°C, respectively. The optimum sealing temperatures corresponded to the transition onset temperature of the films as determined by differential scanning calorimetry (DSC). To my knowledge, voltage-based impulse sealers have not been used in sealing biopolymers. Due to the surface area required to form seals with the thermal sealer, 1.5cm seal width, impulse sealing with a voltage based sealer might be more appropriate for food applications since a narrower seal width can be obtained (0.25cm). The advantage of a narrow seal is that less total film would be used were casings to be

casings to be produced in high volumes, furthermore, the narrower seal would be visually more appealing.

Seal strength values provide general information about the strength of the bond formed between polymer layers during sealing; this information, however, does not provide insight into the chemical characteristics of the bond. By conducting a surface analysis, surface-specific information is obtained that can be valuable for determining bonding mechanisms; this information can also be useful when developing new materials and applications (Rindlav-Westling and Gatenholm 2003). Electron Spectroscopy for Chemical Analysis (ESCA) also referred to as XPS (X-ray photoelectron spectroscopy) provides insight into the surface chemistry of the material tested and has been used as the principal technique for defining interfacial molecular properties associated with adhesive behavior of materials. The use of ESCA allows identification and quantification of all elements except hydrogen and helium (Ratner and Castner, 1997).

Kim and Ustunol (2001c) conducted a unique study where surface chemistry of seals of uncured whey protein based films was investigated. They reported that carbon was the main element of the films followed sequentially by oxygen and nitrogen. Their results indicated that formation of C-O-H and N-C bonds upon heat sealing might be responsible for the mechanism of seal formation. These data provided by Kim and Ustunol (2001c) focused on uncured WPI-based films. Surface chemistry of heat cured WPI-based films have not been investigated using this method. The objectives of this study were to optimize seal strengths of WPI-based films for sausage casing manufacture and to determine their surface chemistry using ESCA.

4.3. MATERIALS AND METHODS

4.3.1. Materials

Whey protein isolate (WPI, Provon 190) was supplied by Glanbia ingredients (Monroe, WI). Glycerol was purchased from J.T. Baker Co. (Phillipsburg, NJ). Sodium hydroxide (2N, NaOH) was purchased from Mallinckrodt Specialty Chemical Co. (Paris, KY). Candelilla wax (CW) was purchased from Strahl and Pitsch Inc. (West Babylon, NY) and magnesium nitrate was purchased from Sigma Chemical Co. (St Louis, MO).

4.3.2. Whey protein isolate film preparation

Figure 4.1 shows a schematic diagram of the experiments conducted under this objective designed to optimize the films for sausage casing manufacture. The levels of the film constituents used were: WPI (5.0% or 5.5% w/v), glycerol (3.3% or 3.6% w/v) and CW (0.8% or 0. 9%, w/v). Two film forming solutions having 5.8% w/v and 6.4% w/v solids contents were developed by dissolving whey protein isolate (WPI) in distilled water, glycerol was added and pH was adjusted to 8.0 using 2N NaOH. The solutions were heated to 90 $\pm$ 2°C with continuous stirring to effectively denature the whey protein. Candelilla wax (CW) was added during heating and allowed to melt into the solutions. The solutions were homogenized for 2 minutes using a Polytron PT 10/35 homogenizer with a PTA 20 TS homogenizing head (Tekmar Co., Cincinnati, OH) at speed 5 (1350 rpm); filtered through a layer of cheesecloth, equilibrated to room temperature for 1.5 h and vacuum degassed at room temperature for 30 min. Films were prepared by pipetting the emulsion (172.6 ml) onto either anodized or non-anodized Teflon® plates (33.0 cm x 45.7 cm). The process of anodizing turns the aluminum into aluminum oxide making it

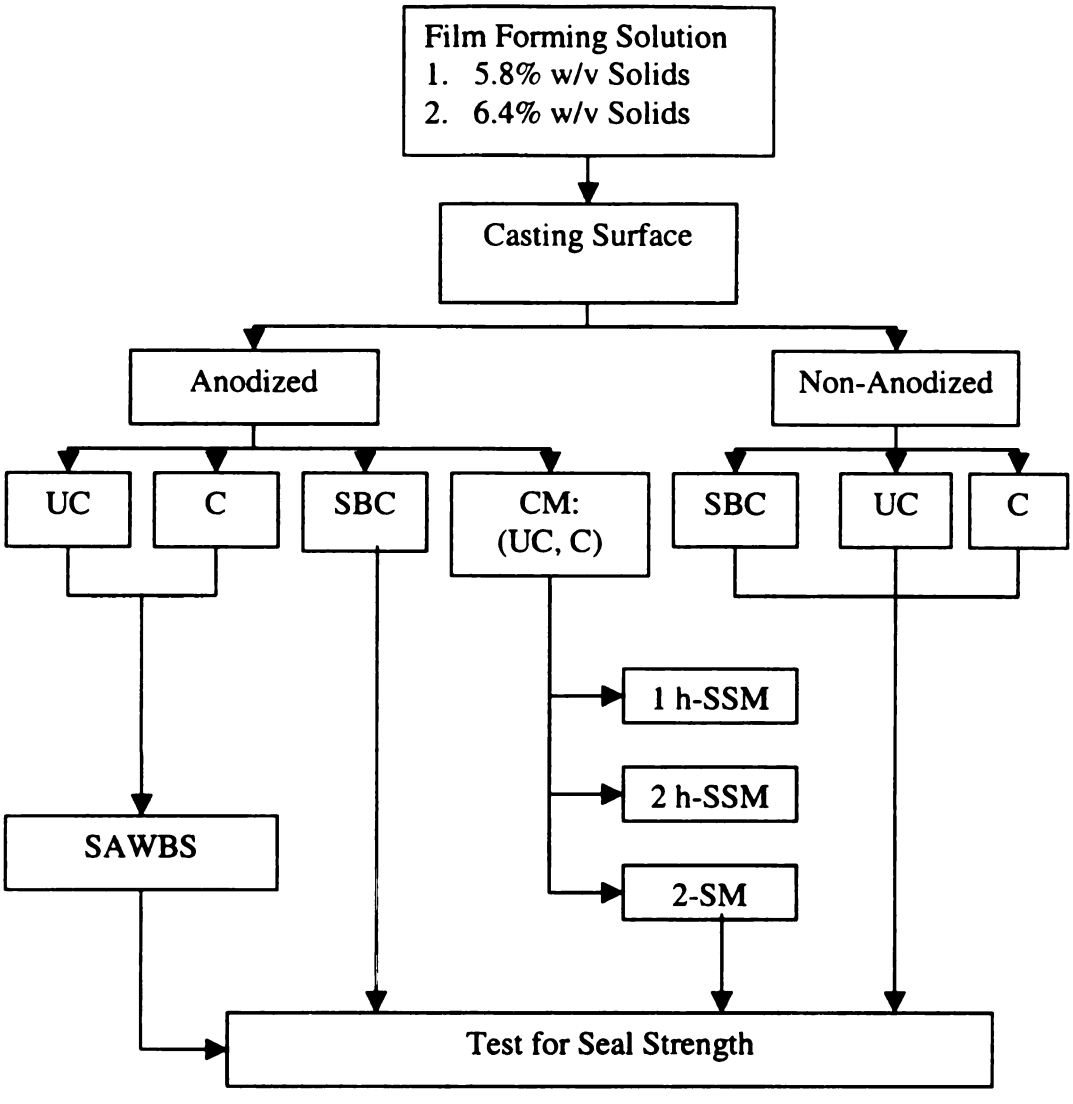


Figure 4.1. Schematic diagram of the seal optimization process for whey protein isolate based films
UC = uncured, C = cured, CM = compression molded, NCM = non-compression molded, SSM = Single stage compression molded (110°C, 1.24 MPa 1 h or 2 h), 2-SM = 2-stage compression molded (90-110°C, 0.99-1.38 MPa, 2-25min), PT = phosphate treatment, SBC = Sealed before curing, SAWBS = seal area wet before sealing

heavier and corrosion resistant. The films were dried at room temperature (23 ± 2°C) and 30 ± 5% relative humidity (RH) for 18 ± 3 h. Dried films were peeled, wrapped in foil and heat cured in a vacuum (30mm Hg) oven maintained at 90°C for 12 h. The films were removed from the oven and peeled from the foil while still warm for ease of

removal. The films were subsequently conditioned at 50% RH, 23°C using magnesium nitrate for 48 h prior to testing.

4.3.3. Compression molding

Whey protein isolate (WPI)-based films were compression molded using a Carver Laboratory Press (Fred Carver, Inc.; Wabash IN). Film sheets (130 x 200 mm) were sandwiched (one at a time) between Teflon® sheets, placed between 2 stainless steel molding plates and inserted between heated plates of the compression compartment of the compression molder. Molding conditions were: 1) single-stage (110°C, 1.24 MPa, 1 h or 2 h) and 2) two-stage compression molding (110°C, 0.99 MPa, 25 min and 110°C, 1.24 MPa, 12 min). 5.8% w/v solids films were used for the compression molded film studies.

4.3.4. Seal strength determination

Two strips (101.6mm x 25.4mm) cut using a Precision Sample Cutter (Thawing Albert Instrument Co., Philadelphia, PA) were sealed together using a voltage based impulse sealer Model-12ASL (Sencorp System Inc., Hyannis, MA). The impulse sealer settings were as follows: pressure: 300kPa, voltage: 22 amps, impulse time: 1.8 seconds and dwell time: 1.4 seconds. Seal strength tests were conducted using an Instron Universal Testing Machine, Model 2401 (Instron Corp., Canton, MA) at 23 ± 2°C and 50% ± 5% RH according to standard ASTM F-88 (ASTM 1997). Each end of the sealed specimen was clamped to the machine and the sealed film was held perpendicularly to the direction of the pull as the seal was tested. The distance between the clamps was 5.08cm and a 1kN static load cell with a speed of 50.8cm/min was used. Seal strength was reported as the maximum force required to cause seal failure and expressed as load (N)/width (m).

4.3.5. Electron spectroscopy for chemical analysis

Surface chemical analysis of uncured, heat cured and compression molded films was conducted using Electron Spectroscopy for Chemical Analysis (ESCA). The unsealed film and the outer and inner seal of sealed films were examined. This was performed to determine the type of bonding that took place during seal formation in the films. The outer seal was observed to make adequate comparisons with the inside of the seal. A PHI 5400 ESCA lab workstation was used (Physical Electronics, Eden Prairie, MN). A 15 mm diameter circular film (sealed or unsealed) was placed in a sample holder (non-lipid oriented side up) and monochromatic X-rays were used as the radiation source. A magnesium anode operated at 300 W with an analyzer pass energy of 33eV was used to collect all spectra and the takeoff angle from the surface to the detector was 45°. An electron kinetic energy analyzer plotted the intensity of the emitted photoelectrons and optimum spot size for the conditions used in the experiments was 1mm diameter aperture.

The bonding scale was calibrated to 284.6eV for the main carbon 1s (C1s; C-H) spectra. Spectra were run at both high and low resolution (wide/survey scan) modes. Curve fitting was used to resolve the ESCA spectra into separate peaks to determine the carbon chemistry detected within the spectral envelope. The spectra were fit with a Lorentzian-Gaussian mix Voigt profile function using a nonlinear least-square program PHI PC Explorer Software multipack (Physical Electronics, Eden Prairie, MN). The resulting curve-fits had an experimental error of approximately 5%. The area under each peak was used to quantify bonds and elemental compositions were obtained from the wide/survey scan spectra, the element and bond quantities were reported in percent of total elements and bonds present.

4.3.6. Statistical analysis

The effect of heat curing (curing), casting surface (surface), total solids content of casting solution (solution) and compression molding (molding) on TS and %E of WPI-based films were determined using the models shown below:

Y = function (curing; surface; solution) (1)

Y= function (curing; compression molding) (2)

Where Y = seal strength, curing = uncured or heat cured, surface = anodized or non-anodized, solution = 5.8 or 6.4% w/v solids and compression molding = single or 2-stage as described in section (4.3.3). The mixed procedure of SAS was used to analyze the data. Treatment means were compared using the least squares means (LSM) and the means were based on 3 replicates (SAS, 1996). ESCA results provided are for only one replicate.

4.4. RESULTS AND DISCUSSION

4.4.1. Seal strengths

Table 4.1 shows the seal strengths of uncured and heat cured films cast from 5.8% and 6.4% w/v solids film forming solutions and cast on non-anodized and anodized surfaces. With the exception of the 6.4% w/v solids solution film cast on a non-anodized surface, uncured films had higher seal strengths than cured films ($p<0.05$). The highest seal strength was observed in uncured 6.4% w/v solids films (173.62 N/m). Because higher seal strengths were observed for uncured films compared to cured films, two additional experiments were conducted to test if seal strength of heat cured films could be

increased. These were: sealing films prior to heat curing and wetting the seal area of uncured and heat cured films prior to sealing.

It was hypothesized that by sealing the films prior to heat-curing, the seal strengths of the heat-cured films would improve. Table 4.2 shows the seal strengths of films that were uncured, heat cured and sealed before curing. Even though the seal strength values of the films sealed prior to heat curing are higher than those of cured films, no statistical difference was detected. The high variation (evidenced by the wide standard deviations) in the seal strengths could explain the non-detection of statistical differences. This variability was seen consistently among the seal strength values of the films. The experiment in which the seal area was wet prior to sealing was based on the hypothesis that the higher seal strengths of uncured films were a result of the higher moisture in these films. Wetting the seal area of uncured and cured WPI-based films did not increase the seal strength of the films (Table 4.3). This treatment, however, decreased the seal strength of uncured films making it similar to that of the cured films. It is possible that the water molecules between the layers being sealed prevented close interaction required to form strong seals. Furthermore, the water had to be evaporated first before bond formation could take place; this too could have affected the strength of the seal formed. Table 4.3 also shows seal strengths of compression molded films. Films compression molded for one hour had the highest seal strength (148.9 N/m). The lowest seal strength was observed for cured films compression molded for two hours (32.09 N/m). Compared to the heat cured films, seal strengths of compression molded films were not different. This indicates that compression molding had a similar effect to heat curing on WPI-based films.

Table 4.1. Influence of casting surface and solids content on seal strengths of whey protein isolate based films

Casting Surface	Casting Solution Solids Content (w/v)	Seal Strength (N/m)	
		Uncured	Cured
Non-Anodized	5.8%	115.33± 77.39[a]	78.18 ± 37.25[b]
	6.4%	79.97 ± 28.68[a]	92.28 ± 29.90[b]
Anodized	5.8%	92.18 ± 39.71[a]	61.56 ± 36.19[b]
	6.4%	173.62 ± 91.43[a]	80.91 ± 51.29[b]

[a-b]Means with different superscripts are significantly different ($p<0.05$); means ± standard deviations; n=3 for all treatments; comparisons are made within each row.

Table 4.2. Influence of casting surface and solids content on seal strengths of uncured, heat cured and sealed before curing whey protein isolate based films.

Casting Surface	Casting Solution Solids Content (w/v)	Seal Strength (N/m)		
		Uncured	Cured	Sealed Before Curing
Non-Anodized	5.8%	115.33± 77.39[a]	78.18 ± 37.25[a]	80.28 ± 37.75[a]
	6.4%	79.97 ± 28.68[b]	92.28 ± 29.90[b]	96.70 ± 30.44[b]
Anodized	5.8%	92.18 ± 39.71[a]	61.56 ± 36.19[a]	54.29 ± 21.56[a]
	6.4%	173.62 ± 91.43[b]	80.91 ± 51.29[b]	142.79 ± 32.46[b]

[a-b]Means with different superscripts are significantly different ($p<0.05$); means ± standard deviations; n=3 for all treatments; comparisons are column-wise within each surface treatment.

Table 4.3. Effect of wetting the seal area before sealing and compression molding on seal strength of whey protein isolate films

Film Treatment	Casting Solution Solids Content (w/v)	Seal Strength (N/m)	
		Uncured	Cured
Anodized (control)	5.8%	92.18 ± 39.71[a]	61.56 ± 36.19[a]
Seal Area Wet Before Sealing	5.8%	67.87 ± 26.70[a]	68.70 ± 63.24[a]
2-Stage Compression Molded	5.8%	93.86 ± 54.01[a]	82.18 ± 71.69[a]
1-Hour Compression Molded	5.8%	101.75 ± 42.04[a]	148.90 ± 49.81[a]
2- Hour Compression Molded	5.8%	91.86 ± 30.96[a]	32.09 ± 22.87[b]

[a-b]Means with different superscripts are significantly different ($p<0.05$); means ± standard deviations; n=3 for all treatments; comparisons are made within each row; compression molding conditions were: single-stage (110°C, 1.24 MPa, 1 h or 110°C, 1.24MPa, 2 h) and two-stage (110°C, 0.99 MPa, 25 min and 110°C, 1.24 MPa, 12 min)

The seal strengths of the films in this study varied from 32.09 N/m to 173.62 N/m. The latter value, obtained for uncured films was in agreement with seal strength values obtained by Kim and Ustunol (2001c) for WPI-glycerol-CW films heat sealed at 130°C for 1 min. Kim and Ustunol (2001c) tested films sealed with a thermal sealer over a temperature range of 110 to 130°C and dwell times of 1 or 3 sec and a pressure of 296 or 445 kPa. The results of this current study indicate that such values are typical and can be expected for uncured WPI-based films since the films tested by Kim and Ustunol (2001c) were also uncured.

One of the possible reasons for the variation in the seal strengths observed in this study may have been the failure mode of the film. Failure mode refers to the manner in which the seal breaks during testing. Meka and Stehling (1997) described three types of seal failure modes; these were "peeling failure", "tearing failure" and a combination of "peeling and tearing failure". During testing of the films in this current study, all three failure modes were observed for different samples as each leg of the sealed film was pulled to determine seal strength. The seal strength measured depended in part on the predominant qualitative failure mode. Some seals peeled open, less strength was obtained for these; others tore, these demonstrated higher seal strength. The way in which the seal opened (failed) however, could not be controlled.

4.4.2. Electron spectroscopy for chemical analysis

Table 4.4 shows the chemical bond composition and concentration of unsealed, outer and inner seals of uncured, heat cured and compression molded WPI-based films as determined by ESCA. Figure 4.2 shows the resolved C1s spectra for the same films. Carbon was the most abundant element and was followed by oxygen. Nitrogen was detected only in unsealed and inner seals of uncured films. These results suggest that during sealing or heat curing, molecular conformations within the protein film change causing certain groups to be less detectable on the surface. This may be due to the groups being buried below the penetration depth of ESCA, which ranges from 5-10 nm. Such changes can be expected because new or additional bonds develop as a result of cross-linking due to heat. These findings are different from those obtained by Kim (2001c) who reported detection of N for both unsealed and sealed uncured WPI-based films.

Table 4.4. Bond composition of unsealed and sealed whey protein isolate based edible films determined with Electron Spectroscopy for Chemical Analysis

Binding Energy, eV	285.0	286	286.5	288.0
Bond Type	C-H, C-C	C-N	C-O-H, C-O-C	C=O
Film	Bond Concentration, %			
Uncured	45.34	1.11	ND	2.09
Uncured outer seal	85.29	ND	0.29	0.04
Uncured inner seal	58.05	0.14	ND	0.40
Cured	87.03	ND	0.24	0.05
Cured outer seal	84.98	ND	0.40	ND
Cured inner seal	71.21	ND	0.67	0.12
Molded	86.27	ND	0.30	0.05
Molded outer seal	84.93	ND	0.36	0.04
Molded inner seal	82.59	ND	0.45	0.08

C = Carbon, O = Oxygen, N = Nitrogen, H= Hydrogen, Molded = compression molded

[1]% = Percentages represent percentage of total element composition, quantities do not add up to 100 since some elements were not detected

ND = Not detectable

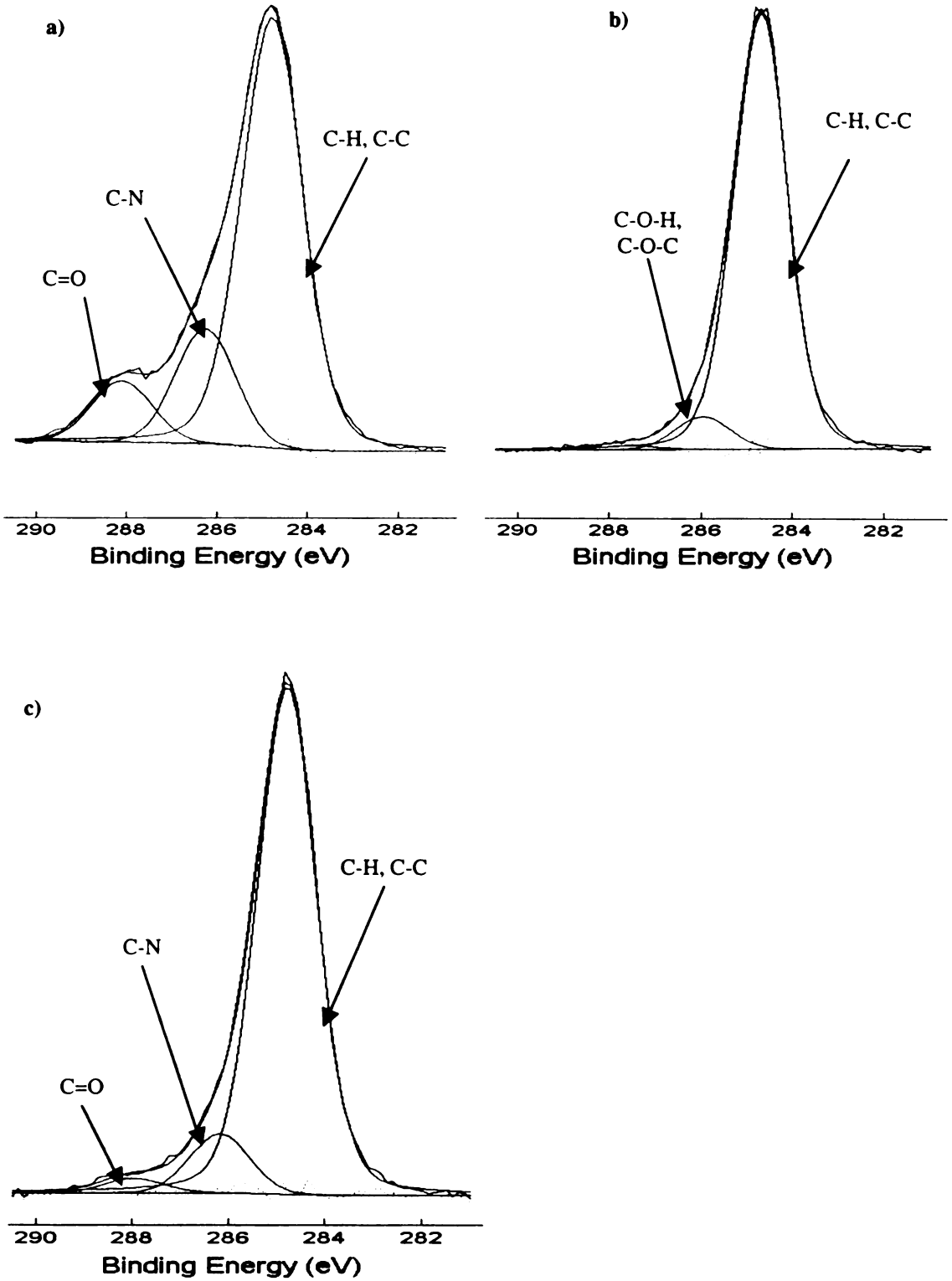


Figure 4.2a. Representative Electron Spectroscopy for Chemical Analysis spectra of whey protein isolate based films. a) uncured b) uncured outer seal c) uncured inner seal

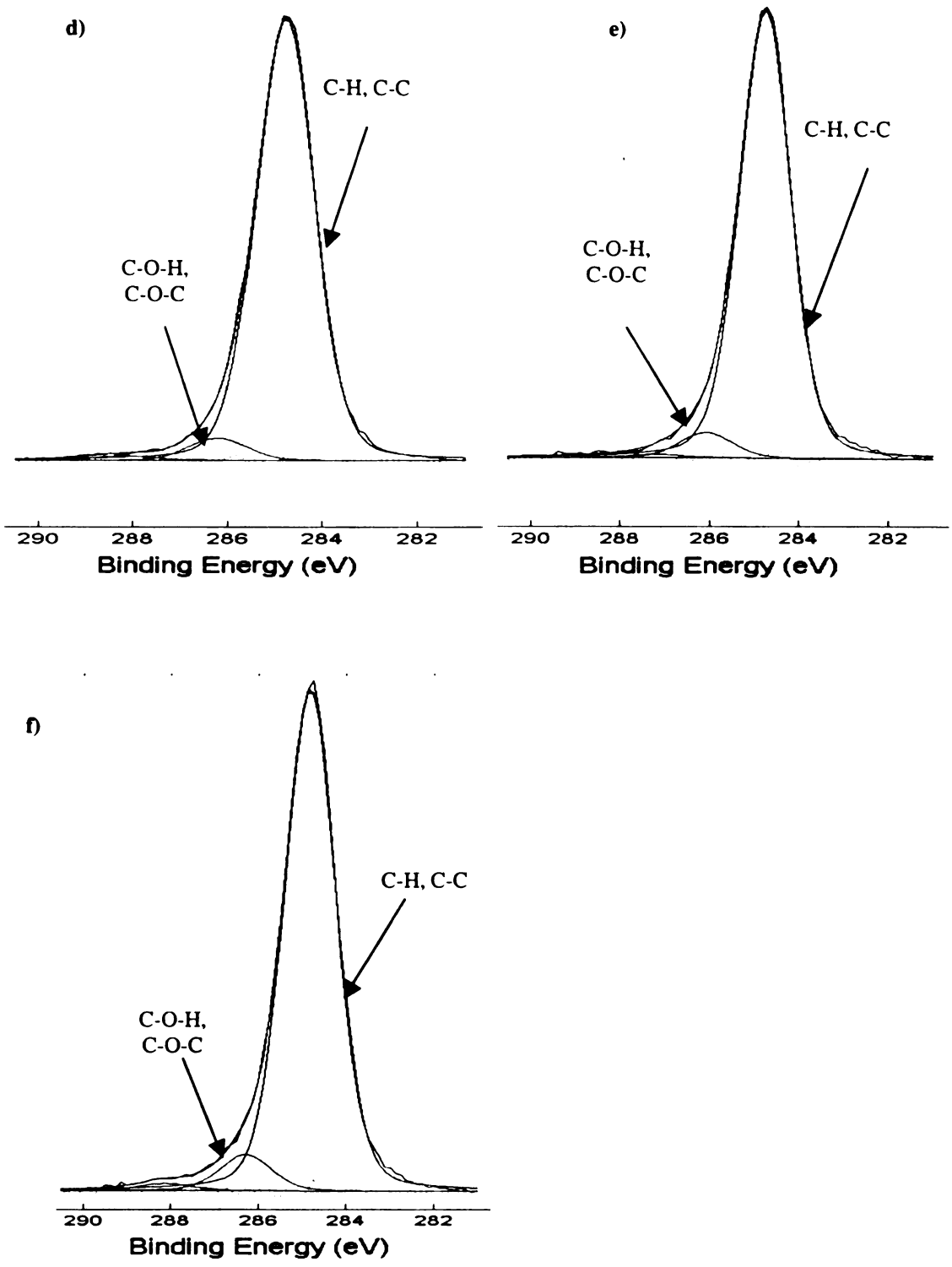


Figure 4.2b. Representative Electron Spectroscopy for Chemical Analysis spectra of whey protein isolate based films.d) cured e) cured outer seal f) cured inner seal

The abundance of C can be attributed to the protein, wax and glycerol used to make the films and is expected due to the total organic matter inherent in these films. The C1s spectra consisted of three components for heat cured, sealed and compression molded films whose binding energies ranged from 284.8eV to 288.0eV. These were C-H or C-C (285.0eV), C-O-H or C-O-C (286.5eV) and C=O (288.0eV). Uncured films had an additional component (C-N), which had a binding energy of 286.0 eV. Resolving of the C1s spectra revealed peaks representing bonds present within each film (Figure 4.2). Once the C1s spectra was resolved, bond concentrations were quantified based on the area under the curve for each peak and the results showed that the hydrocarbon C-H and C-C bonds constituted the bulk of the bonds in these films and they increased with heat curing and compression molding (45.34% in uncured films vs. 87.03% in heat cured films). In surface studies of starch, amylose and amylopectin films, Rindlav-Westling and Gatenholm (2003) attributed the presence of this bond to the presence of proteins and lipids contained within potatoes. They tested starch films prepared by solvent casting of starch-water solutions made of potato starch, amylose and amylopectin. Examinations of the films using ESCA showed the presence of the C-C bond, which they reported, did not originate from starch but from proteins and lipids. Russel and others (1987) stated that low binding energy (285 eV), indicates the presence of lipids or proteins within the matrix of films tested. This is consistent with the formulation protocol of films used in this study, which consisted of WPI and CW.

It was also apparent that compression molding had a similar effect to heat curing. Similar bonding was found between films put through both of these treatments. Uncured unsealed films did not contain the preponderance of C-O-H or C-O-C bonds compared to

the cured sealed films and uncured sealed film. These bonds were also present in all heat-cured and compression molded films. This indicates that the C-O-H or C-O-C bonds are important for adequate seal formation, as shown by the uncured sealed film. It also demonstrates that these bonds are formed during heat curing and compression molding. These results are consistent with adhesion reports by Wu and others (1995) who reported that C-O-H components on the surfaces of polyolefins participated in hydrogen bonding across the interfaces of these polymers. Hydrogen and covalent bonds are reported to play important roles in interfacial interactions required for adequate bond strengths (Urban 1993, Misra 1994). Hydrogen bonding in proteins is well documented (Cheftel and others 1985, Rasco and Zhong, 2000). Hydrogen bonds appear between carbonyl (C=O), amine (NH) or hydroxyl (OH) groups of the polypeptide chains. Together with hydrophobic, disulfide, electrostatic and dipole interactions, hydrogen bonds help to stabilize protein structures (Cheftel and others 1985, Rasco and Zhong, 2000). The findings of this study are also consistent with those of Kim and Ustunol (2001c) who reported that formation of C-O-H bonds was important for obtaining adequate seals in WPI-based films. Kim and Ustunol (2001c) proposed a model for the nature of the hydrogen bonding that takes place between plasticizer and plasticizer; plasticizer and protein; as well as between protein and protein upon heat-sealing of WPI-based films.

In this study nitrogen bonding was only detected in uncured films and there was a significant decrease in this bond after sealing, this is clearly seen in the inner seal bond composition of the uncured film (Table 4.4). A possible explanation for the non-detection of C-N bonds after sealing may be the formation of new bonds or changes in

molecular conformation that may result in these groups becoming buried below ESCA penetration levels. This phenomenon, however, requires further research. An assessment of carbonyl (C=O) bonds demonstrated that uncured films had the highest concentration of C=O bonds (2.09%) among all the samples tested. Inner seal of uncured films had the second highest concentration of this bond (0.40 %); these bonds were not detected in outer seals of cured films. The reduction of the C=O bond in sealed, cured and compression molded films may possibly be due to the participation of these bonds in the formation of hydrogen bonds. The model proposed by Kim and Ustunol (2001c) shows hydrogen bonding taking place between carbonyl and amine groups. Formation of the hydrogen bonds would cause reduction of the carbonyl (C=O) bonds and an increase in C-O-H and C-O-C bonds. This would explain the increase observed in the concentration of these bonds. This would also be consistent with the hydrogen bonding mechanism presented by Cheftel and others (1985).

The presence of N and the C-N bond in the unsealed and inner seal of the uncured films suggests the possibility of protein cross-linking taking place between these films upon sealing. This would be consistent with the protein-protein model proposed by Kim (2001c) for bonding between lysine and asparagine and; lysine and glutamine during heat sealing. These amino acids are abundant in β-lactoglobulin and α-lactalbumin, the main components of whey proteins. This may explain the higher seal strengths observed for uncured films compared to those of heat cured films, for example 173.62 N/m vs. 80.91 N/m for 6.4% w/v solids films (Table 4.1). The absence of N and abundance of hydrocarbon groups on the heat-cured and compression molded films suggests that bonding in these films is likely taking place between hydrophobic groups associated with

wax molecules; whose relative concentration may have increased on the film surface during the heating processes. This phenomenon would not be unlikely as lipids have a lower density than proteins.

4.4. CONCLUSIONS

Seal strengths of uncured WPI-based films were higher than those of heat cured and compression molded films. Seal strengths could not be optimized by sealing films prior to heat curing or wetting the seal area of uncured and heat cured films prior to sealing. Additional experiments were conducted to test the performance of sealed tubular WPI-based casings under sausage manufacturing conditions. Results of these preliminary experiments are shown in Appendix II. C-O-H and C-O-C bonds were prevalent in heat cured, sealed and compression molded films. C-N bonds were only detected in uncured films.

SUMMARY

In this research, experiments were conducted to investigate the potential of utilizing whey protein isolate (WPI)-based films in a sausage manufacturing process and thereafter optimizing the mechanical and sealing properties of the films for development of a sausage casing. Further experiments were conducted to determine the surface and interfacial chemical properties of unsealed and sealed films.

The results showed that although WPI-based films remained intact, the TS of the films decreased with increasing temperature, time and relative humidity of the sausage manufacturing process. Sausage manufacturing conditions did not affect %E. Compared to commercial collagen casings, WPI-based films had similar %E but lower TS. Mechanical property optimization experiments showed the following:

- Heat-curing increased TS of uncured films and did not decrease %E, the presence of wax in the film matrix may have contributed to mobility within the protein film structure, thus causing the films to retain their flexibility.
- Compression molding increased TS of uncured films and the TS values were similar to those obtained by heat-curing. These results indicate that compression molding can be used to heat-cure films for a lesser time than vacuum heat-curing.
- The increase in TS resulting from heat curing and compression molding was not sufficient to give WPI-based films TS equivalent to collagen films. This indicates that TS of WPI-based films needs to be increased further to enable these films to be used in sausage manufacture

- Through ESCA we discovered that heat curing, sealing and compression molding increased carbon concentration on the surface of the films and N became undetectable.

FUTURE RESEARCH

In order to manufacture sausage casings with the WPI-based edible films, further work needs to be done to improve their mechanical properties, particularly tensile strength under increased relative humidity (RH) conditions. Reformulation of the film forming solution by incorporating materials such as cellulose might be one way to increase the strength of these films while increasing their resistance to increased RH. Furthermore, I suggest that instead of using solvent casting for film formation, it might be better to use compression molding, because variables like thickness can be controlled during the film formation process. In order to reduce problems that may be encountered with sealing when developing tubular casings from the WPI-based films, I recommend that investigation be made into forming the sausage casings using extrusion; in this way a continuous casing would be obtained. Research is also required to precisely determine the cause of the non-detection of C-N bonds after sealing, heat curing and compression molding of WPI-based films.

APPENDIX I

PRELIMINARY STUDIES ON POST-TREATMENT OF WHEY PROTEIN ISOLATE-BASED FILMS WITH PHOSPHATE SOLUTIONS

Materials

Whey Protein Isolate (WPI, Provon 190) was supplied by Glanbia ingredients (Monroe, WI). Glycerol was purchased from J.T. Baker Co. (Phillipsburg, NJ). Sodium hydroxide (2N, NaOH) was purchased from Mallinckrodt Specialty Chemical Co. (Paris, KY). Candelilla wax (CW) was purchased from Strahl and Pitsch Inc. (West Babylon, NY) and magnesium nitrate was purchased from Sigma Chemical Co. (St Louis, MO) and phosphate solution was obtained from Butcher and Packer Supply Company (Detroit, MI).

METHODS

Whey protein isolate based film preparation

Two film forming solutions having 5.8% w/v and 6.4% w/v solids contents were developed by dissolving whey protein isolate (WPI) in distilled water, glycerol was added and pH was adjusted to 8 using 2N NaOH. The solutions were heated to 90 $\pm$ 2°C with continuous stirring to effectively denature the whey protein. Candelilla wax was added during heating and allowed to melt into the solutions. The levels of the film constituents used were: WPI (5.0% or 5.5% w/v), glycerol (3.3% or 3.6% w/v) and CW (0.8% or 0. 9%, w/v). The solutions were homogenized for 2 minutes using a Polytron PT 10/35 homogenizer with a PTA 20 TS homogenizing head (Tekmar Co., Cincinnati,

OH) at speed 5; filtered through a layer of cheesecloth, equilibrated to room temperature for 1.5 h and vacuum degassed at room temperature for 30 min. Films were prepared by pipetting the emulsion onto either anodized or non-anodized Teflon® plates. The films were dried at room temperature (23 ± 2°C) and 30 ± 5% relative humidity (RH) for 18 ± 3 h. Dried films were peeled, wrapped in foil and heat cured in a vacuum (30mm Hg) oven maintained at 1) 80°C for 24h or 2) 90°C for 12 h. The films were removed from the oven and peeled from the foil while still warm for ease of removal. The films were subsequently conditioned at 50% RH, 23°C using magnesium nitrate for 48 h prior to testing.

Film thickness

Films were preconditioned at 23°C, 50% RH using magnesium nitrate for a minimum of 8 h to enable ease of handling and cutting into strips (101.6mm x 25.4mm) using a Precision Sample Cutter (Thawing Albert Instrument Co., Philadelphia, PA). Film thickness was determined using a TMI model 549M micrometer (Testing Machines, Inc. Amityville, NY). Five different measurements were taken in random locations within the filmstrip test area and the mean values were used for calculations of mechanical properties.

Phosphate treatment

Uncured and cured film strips were post-treated by dipping them in a potassium phosphate solution for 10 s. Food grade potassium phosphate solution obtained from Butcher and Packer Supply Company (Detroit, MI) was used. The treatment concentrations used were: 0%, 12.5%, 25% and 50% (v/v). Following dipping in the phosphate solution, the films became brittle and cracked easily. The phenomenon

causing the film brittleness was investigated as follows: 1) ethyleneglycol-bis-(β-amino-ethyl-ether)-N,N'-tetra acetic acid (EGTA) was added to the films forming solutions after WPI and glycerol had been dissolved. The concentrations of EGTA were 2.5, 5, 10, 15, 20 or 25 mM in the film forming solutions 2) potassium phosphate solution (9.2% v/v) was added to the film forming solutions after the addition of EGTA 3) the solutions were then heated as described above.

Statistical analysis

The effect of heat curing (curing), total solids content of casting solution (solution) and phosphate post-treatment (phosphate post-treatment) on TS and %E of WPI-based films were determined using the models shown below:

Y = a linear function (heat curing; solution; phosphate post-treatment) (7)

Where Y = TS or %E, curing = uncured or heat cured, solution = 5.8 or 6.4% w/v solids and phosphate post-treatment = 12.5, 25, 50% w/v phosphate concentration. All comparisons were also made to collagen films. The mixed procedure (PROC MIXED) of SAS was used to analyze the data. Treatment means were compared using the least squares means (LSM) and the means were based on 3 replicates.

RESULTS

Table I.1 shows the mechanical properties (TS and %E) of films post-treated with potassium phosphate solution (0, 12.5, 25 and 50% v/v). Uncured films had lower TS and %E than cured films for all treatments, comparisons to untreated films showed that TS of phosphate treated films were similar to non-treated films; however, %E was

significantly reduced ($p<0.05$). Phosphate treated films became brittle and, thus, no sealing tests could be performed on these films. These results are different from those of Otaigbe and Adams (1997) who showed that addition of polyphosphate fillers to SPI films improved TS while reducing water absorption. Polyphosphate fillers were added prior to compression molding at 10, 20 and 30% (wt of SPI) and were effective up to 20% w/w. Results of Otaigbe and others (1997) showed that increases in polyphosphate concentration were not effective in increasing the TS. The successful use of polyphosphate fillers by these researchers suggests that similar treatments may be applied to WPI-based films and may yield more successful results instead of the post-treatment method used in the current study.

Table I.1. Mechanical properties of films post-treated with potassium phosphate solution

Film Solution Solids (%w/v)	Phosphate Solution (%)	TS (MPa)		%E	
		Uncured	Cured	Uncured	Cured
5.8	0	2.71 ± 1.31^{a}	7.16 ± 2.33^{b}	20.52 ± 18.66^{b}	36.89 ± 10.72^{d}
	12.5	2.88 ± 1.16^{a}	5.19 ± 3.42^{b}	24.59 ± 9.47^{c}	30.66 ± 5.48^{d}
	25.0	2.91 ± 1.34^{a}	5.35 ± 3.21^{b}	10.21 ± 4.35^{c}	21.59 ± 13.15^{d}
	50.0	2.28 ± 1.26^{a}	4.45 ± 2.74^{b}	$7.58 \pm 3.56_{c}$	20.47 ± 13.74^{d}
6.4	0	3.01 ± 0.62^{a}	3.90 ± 1.71^{b}	21.62 ± 10.44^{c}	9.63 ± 8.70^{d}
	12.5	2.50 ± 1.51^{a}	5.38 ± 2.83^{b}	27.78 ± 7.35^{c}	20.44 ± 9.18^{d}
	25.0	2.79 ± 1.68^{a}	4.86 ± 2.52^{b}	10.77 ± 3.81^{c}	18.05 ± 5.42^{d}
	50.0	2.36 ± 1.14^{a}	4.63 ± 1.92^{b}	6.33 ± 3.16^{c}	31.90 ± 19.35^{d}
Collagen		13.29 ± 3.47^{e}		31.78 ± 4.88^{e}	

[a-e]Means followed by the same superscript are not significantly different ($p<0.05$), comparisons are made within the same column, n=3 for all treatments, TS = Tensile strength, %E = Elongation (%).

The presence of some bonding interaction between the phosphate and the protein was demonstrated by the phenomenon of the films becoming brittle. The behavior of the phosphate in the film-forming solution was thereafter investigated. Addition of the potassium phosphate solution to the film-forming solution resulted in gel formation upon heating of the solution. One possible mechanism of this gel structure is the formation of calcium-phosphate bridges. To test this hypothesis, ethyleneglycol-bis-(β-amino-ethyl-ether)-N,N'-tetra acetic acid (EGTA) was added to the film forming solution to chelate calcium. The addition of EGTA, however, was not successful in reducing or preventing gelation. This indicated that the interaction between the whey proteins and phosphates was not a result of calcium-phosphate bridges and suggests that there may be a different interaction taking place between whey proteins and phosphates that we are not aware of. Further studies are required to determine precisely the nature of the interaction between phosphates and WPI.

APPENDIX II

PRELIMINARY STUDIES OF POLISH SAUSAGE MANUFACTURE USING WHEY PROTEIN ISOLATE BASED FILMS AND COLLAGEN CASINGS

Materials

Whey Protein Isolate (WPI, Provon 190) was supplied by Glanbia ingredients (Monroe, WI). Glycerol was purchased from J.T. Baker Co. (Phillipsburg, NJ). Sodium hydroxide (2N, NaOH) was purchased from Mallinckrodt Specialty Chemical Co. (Paris, KY). Candelilla wax (CW) was purchased from Strahl and Pitsch Inc. (West Babylon, NY) and edible collagen casings (inside diameter, 32mm) were obtained from The Brechteen Co. (Chesterfield, MI).

METHODS

Whey protein isolate based film preparation

Figure 4.1 shows a schematic diagram of the experiments conducted under this objective designed to optimize the films for sausage casing manufacture. Two film forming solutions having 5.8% w/v and 6.4% w/v solids contents were developed by dissolving whey protein isolate (WPI) in distilled water, glycerol was added and pH was adjusted to 8 using 2N NaOH. The solutions were heated to 90 $\pm$ 2°C with continuous stirring to effectively denature the whey protein. CW was added during heating and allowed to melt into the solutions. The levels of the film constituents used were: WPI (5.0% or 5.5% w/v), glycerol (3.3% or 3.6% w/v) and CW (0.8% or 0. 9%, w/v). The solutions were homogenized for 2 minutes using a Polytron PT 10/35 homogenizer with a

PTA 20 TS homogenizing head (Tekmar Co., Cincinnati, OH) at speed 5; filtered through a layer of cheesecloth, equilibrated to room temperature for 1.5 h and vacuum degassed at room temperature for 30 min. Films were prepared by pipetting the emulsion onto either anodized or non-anodized Teflon® plates. The films were dried at room temperature (23 ± 2°C) and 30 ± 5% relative humidity (RH) for 18 ± 3 h. Dried films were peeled, wrapped in foil and heat cured in a vacuum (30mm Hg) oven maintained at 1) 80°C for 24h or 2) 90°C for 12 h. The films were removed from the oven and peeled from the foil while still warm for ease of removal. The films were subsequently conditioned at 50% RH, 23°C using magnesium nitrate for 48 h prior to testing.

Compression molding

Whey protein isolate-based films were compression molded using a Carver Laboratory Press (Fred Carver, Inc.; Wabash IN). Film sheets (130 x 200 mm) were sandwiched (one at a time) between Teflon® sheets, placed between 2 stainless steel molding plates and inserted between heated plates of the compression compartment of the compression molder. Molding conditions were: 1) single-stage (110°C, 1.24 MPa, 1 h or 2 h) and 2) two-stage compression molding (110°C, 0.99 MPa, 25 min and 110°C, 1.24 MPa, 12 min). 5.8% w/v solids films were used for compression molding.

Manufacturing of tubular whey protein-based casings

Figure II.1 shows tubular WPI-based casings developed by heat-sealing of the films. Heat-sealed tubular casings were manufactured to resemble sausage casings and commercial collagen casing dimensions were used (32mm). A sheet of whey protein film (35mm x 200mm) was rolled and heat-sealed on two sides using an impulse sealer Model-12ASL (Sencorp System Inc., Hyannis, MA). The impulse sealer settings were as

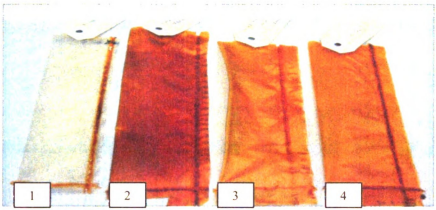


Figure II.1. Whey protein isolate based edible films heat-sealed to form sausage casings 1 = uncured film, 2 = compression molded film, 3 = film sealed before heat curing, 4 = heat cured film
follows: pressure: 300kPa, voltage: 22 amps, impulse time: 1.8 seconds and dwell time: 1.4 seconds. Casings were made with uncured, heat cured and compression molded films

Sausage manufacturing

Figures II.2 and II.2 show stuffing of the Polish sausage and the smokehouse in which the sausages were cooked, respectively. Sausage was made using turkey meat and seasoned with Polish sausage seasoning (Legg's sausage seasoning, moderncure (156ppm) with salt and erythorbate (500ppm)). The mixture was stuffed into the casings using a hand stuffer (VOGT-deal, Chicago IL, USA). The cooking schedule used is shown in Table II.1.

Table II.1. Polish sausage smokehouse schedule

Stages	Time, min	Internal Temperature, °C	Dry Bulb, °C	Wet Bulb, °C	Smoke Source, Natural
1	20	0	60	43	off
2	20	0	71	52	on
3	15	0	77	57	on
4	90	72	82	77	off
Shower		32	1 min on	1 min off	

Figure II.2. Hand stuffing of sausage into whey protein isolate based edible sausage casings

Figure II.3. Sausage stuffed in whey protein isolate based edible casings (flat on rack) and collagen casings (hanging on sausage stick) in smokehouse

RESULTS

Figure II.4 shows the external appearance of sausages stuffed in experimental WPI-based edible casings and commercial collagen casings used as control. Although sausage was successfully stuffed into the casings, the casings were very delicate, indicating that the sausage casings still needed further improvement to enable them to

withstand sausage manufacturing conditions. The inadequacy of WPI-based casings was demonstrated by tearing of the seals due to slight increases in the stuffing pressure or filling speed. The best-stuffed casings developed in this experiment were the heat cured films: 5.8%w/v solids, 6.4% w/v solids and the 5.8%w/v film sealed-before heat curing. The uncured and compression molded films did not perform well as they took up a lot of moisture from the meat. This was evidenced by moisture observed on the surface of the film immediately after stuffing. After cooking, the casings that were well stuffed had acceptable appearance while loosely stuffed casings were shriveled and not appealing due to air pockets between the meat and the casing. Uncured casings were shriveled and unappealing; they were also very pale in color. The collagen-cased sausage remained the best-stuffed and best-looking casings.

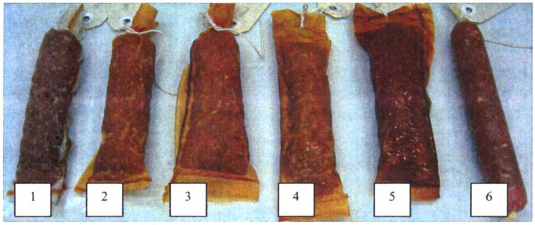


Figure II.4. Sausages made from various stuffed whey protein isolate based edible casings
1 = Uncured film 2 = 5.8% w/v solids film 3 = 6.4% w/v film 4 = film sealed before curing 5 = compression molded 6 = collagen casing

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