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DEVELOPMENT, EVALUATION, AND APPLICATION OF CASEIN-BASED EDIBLE FILMS

By

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ABSTRACT

DEVELOPMENT, EVALUATION, AND APPLICATION OF CASEIN-BASED EDIBLE FILMS

By

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This study was conducted to determine the effect of protein type, plasticizer type, and protein to plasticizer concentration on properties of casein-based films, and then applying films to a food system. Films were produced from lactic acid or rennet precipitated casein, and either sorbitol or glycerol, mixed in distilled water. A lactic acid casein and sorbitol film and a lactic acid casein and glycerol film were used to wrap processed cheese slices, with low density polyethylene (LDPE) wrapped and unwrapped samples used as controls. Films were tested for barrier properties, water vapor and oxygen permeability, and mechanical properties, elongation and tensile strength. The cheese slices and films were tested for moisture content and color change over a 30 day storage period (2.2°C, 88% relative humidity).

Films produced displayed good oxygen barrier properties, but poor water barrier properties compared to synthetic films. Films made with sorbitol exhibited significantly better ($p<0.05$) water barrier and tensile properties than those made with glycerol. Higher protein concentrations also produced stronger films. Processed cheese slices wrapped in casein-based films lost a significant amount of moisture ($p<0.05$) as compared to the LDPE wrapped control slices, which was the cause of a significant color change ($p<0.05$) of the cheese slices. However, moisture lost by the cheese slices was retained in the casein-based films, shown by a large increase in moisture content of these films.

Dedicated to my Parents, Lyle and Lois Ann Chick,

for all their love, support, patience, and

raising me to be the person I am today

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INTRODUCTION

In 1909 Dr. Leo Hendrick Baekland reacted phenol and formaldehyde together to form a polymer which was called Bakelite, and this is known as the first synthetic plastic. Soon to follow was the development of many other new synthetic polymers, like cellulose acetate and polyvinyl chloride in 1927. Polyethylene, the major food packaging plastic, was developed in 1935 by Imperial Chemical Industries in England. The ability to extrude plastic into films was developed in the mid 1940's (Hanlon, 1992). As these synthetic polymers have been further developed to perform useful, packaging and other, functions their use has become widespread. One possible alternative to synthetic polymers that has gained increased attention, due to their value added properties they can impart, is the use of edible films.

Edible films have actually been in use for centuries, for example, the coating of fruit with wax or coating food with lard for longer preservation. There are a number of advantages to be gained through the use of edible films. First, they can enhance functional and nutritional properties of a food. They can be used to protect small pieces or portions of food, or can be used inside heterogeneous foods to separate components. Finally, since they are edible there is little to no waste generated (Guilbert, 1986).

Edible films will probably never be able to replace the qualities that synthetic materials possess, but they still can perform many of the same functions to a lesser extent.

The functions desirable in these films consist of lowering the migration of moisture, fats, and oils. They also decrease the transport of gases like oxygen and carbon dioxide, while forming a barrier against the contamination from outside microorganisms (Kester and Fennema, 1986; Donhowe and Fennema, 1994).

The formation of edible films has been accomplished using high molecular weight polymers, which are necessary in order to form a polymer matrix with enough cohesive strength. The types of high molecular weight polymers used in the making of edible films fall into three categories, being hydrocolloids, lipids, and a composite of both a hydorcolloid and lipid. Lipids used are fatty acids, fatty alcohols, or a combination of both, common types being acetoglycerides, surfactants, and waxes. Hydrocolloids used can be either proteins or polysaccharides, common ones being corn, soy, wheat, and milk proteins or pectin, starch, and cellulose derivatives (Kester and Fennema, 1986).

In this research we will develop edible films using casein from milk. Casein was chosen because it is abundant, inexpensive, and are extensively used in the production of adhesives and coatings. Their functional properties make them very suitable for film production, of which they have not been as extensively studied as whey proteins from milk. The objectives of this research will be to develop optimal formulations and processes for the formation of these casein edible films. These films will be comprised of either rennet or lactic acid precipitated casein, and either sorbitol or glycerol as a plasticizer. Once the films are developed they will be tested for their mechanical properties (tensile strength and elongation) and barrier properties (oxygen and water permeability). Comparisons will be made to determine the effect of protein type, plasticizer type, protein to plasticizer concentration. Then compare these properties with

those of other protein-based films and synthetic polymers whose properties are known. The casein-based films with the best overall properties will then be selected for further evaluation in an actual food system to determine their effectiveness as an alternative packaging material.

LITERATURE REVIEW

Formation of Edible Films and Coatings

There are three basic steps usually followed in the formation of a protein edible film or coating. The mixing of the film forming constituents in the solvent must be done to obtain a dispersion of the high molecular weight polymer. This is followed by casting a thin layer of the film forming solution onto a smooth level surface or the food item in the case of coatings. This then undergoes a drying process to allow the solvent to evaporate, allowing the protein to from a matrix and subsequently the coating or free standing film (Cuq *et al.*, 1995).

Components of edible films

A high molecular weight polymer is the one basic requirement for the formation of an edible film or coating. This is needed because films with enough cohesive strength require long chain polymeric structures (Banker, 1966). Two types of high molecular weight polymers used in the formation of edible films and coatings, which are hydrocolloids and lipids. There are two categories of hydrocolloids used, polysaccharides and proteins. The polysaccharides consist of starches, gums, and modified starches. These include alginate, carrageenan, amylose, and cellulose derivatives. Proteins used in edible films and coatings include corn, soy, wheat, collagen, peanut, and milk protein among others (Donhowe and Fennema, 1994; Kester and Fennema, 1986). The lipids fall into two categories, neutral lipids of glycerides that are esters of glycerol and fatty acids, and waxes which are esters of long chain monohydric alcohols and fatty acids (Hernandez,

1994). Acetylated monoglycerides, natural waxes, and surfactants are the common lipids used in the manufacturing of edible films and coatings (Kester and Fennema, 1986; Hernandez, 1994). These have included lauric, oleic, and stearic acid, carnauba, candelilla, and beeswax, and corn, soybean, and palm oil to name a few.

A solvent system is often used in the formation of edible films and coatings, usually when a hydrocolloid or a composite film is being produced. This makes it possible to solubilize and spread the high molecular weight polymer into a thin layer. The two primary solvents used for these are water and ethanol (Kester and Fennema, 1986).

There are a number of additives that can also be incorporated into the film or film forming solution that alter the properties of the edible film or coating. There purpose is to impart more desirable properties to the film or coating, or to give an added value to the food system. These additives can include plasticizers, crosslinkers, vitamins, antioxidants, flavors, colors, and antimicrobials (Donhowe and Fennema, 1994; Guilbert, 1986).

Plasticizers are widely used in hydrocolloid and composite films. These reduce brittleness and increase flexibility by interfering with intermolecular bonding between adjacent polymer chains (Koelsch, 1994; Guilbert, 1986; Kester and Fennema, 1986). Common plasticizers used in edible films and coatings are glycerol, polyethylene glycol, sorbitol, and sucrose. Crosslinkers have been used to impart an increase in cohesive strength by enhancing intermolecular bonding. These have included such things as transglutaminase, tannic acid, and formaldehyde. Formaldehyde, not being edible imposes some limits on it's use for edible packaging. Antioxidants are incorporated to prolong the food degradation by way of oxidation. Ascorbic acid, citric acid, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) are commonly used food antioxidants.

Antimicrobials are used to hinder the growth of microbes, that can cause spoilage of the food product. These include sorbic acid and potassium sorbate among others.

Processes of edible film and coating formation

One of several processes can be used to form an edible film or coating. These include solidification of melt, coacervation, and solvent removal. Solidification of melt is the common process by which lipid films and coatings are produced. This involves the melting of the lipid followed by it's subsequent cooling to resolidify (Donhowe and Fennema, 1994). Coacervation is the separation of the film forming material from solution by heating, changing pH, adding solvents, or changing the charge. This can be simple, where only one high molecular weight polymer is involved, or complex, where two oppositely charged high molecular weight polymers are used (Donhowe and Fennema, 1994; Kester and Fennema, 1986). The most common process used to form hydrocolloid edible films is by solvent removal. In this process the film forming constituents are dispersed in an aqueous phase, which then undergoes a drying process to remove the solvent (Donhowe and Fennema, 1994).

Casting involves spreading the film forming solution in a thin layer, so evaporation of the solvent and formation of the film can occur. Numerous surface types have been used to cast edible films. The requirements for these surfaces is that it be smooth and level, it is able to contain the film forming solution during drying, and that the film is able to be peeled intact from it's surface after drying. Materials used for casting protein films have included glass, teflon (polytetrafluoroethylene), polystyrene, plexiglass (polymethacrylate), polyethylene (PE), and polyvinyl chloride (PVC).

A glass surface has been successful for the production of cereal protein films (Rayas, 1996; Gennadios *et al.*, 1993a). PE, plexiglass, and PVC have also been used for wheat gluten films, while PE and teflon have been used with soy films (Herald *et al.*, 1995; Gontard *et al.*, 1992; Redl *et al.*, 1996; Stuchell and Krochta, 1994; Brandenburg *et al.*, 1993). Teflon has been used in the production of caseinate, whey protein, and non-fat dry milk films. Polystyrene has also been successful with the caseinate and the whey protein films (Banerjee and Chen, 1995). Whey protein films have also been cast on plexiglass and non fat dry milk films have been cast on high density polyethylene and teflon surfaces (McHugh *et al.*, 1993; Maynes and Krochta, 1994).

Properties of Films Important for Food Applications

Barrier

The quality of many food products is dependent on their loss of or exposure to vapors and gases. These include water (H_2O), oxygen (O_2), carbon dioxide (CO_2), or volatiles (flavors, antioxidants, etc.). One of the roles of polymers used in packaging is to control the migration of these gases and vapors (mass transport) into or out of the package.

The rate at which these vapors or gases pass through a polymer is known as permeation. There are three steps involved in the action of permeation, adsorption of the vapor or gas into the polymers surface, followed by it's diffusion through the polymer, and finally by it's desorption through the opposite surface (Sperling, 1992; Birley *et al.*, 1992). This is expressed by the equation:

$$P = D \times S$$

where P is the permeability coefficient, D is the diffusion coefficient, and S is the solubility constant. Solubility is based on the fact that like dissolves like, so gases and vapors with similar solubility parameters to the particular polymer will dissolve more easily into the polymer. Henry's law expresses this action as:

$$C_D = S_P$$

where C_D is the dissolved equilibrium concentration, and S_P is the gas solubility constant. This expression holds true if S_P is a linear function of the volumetric proportion of the amorphous phase, but a temperature dependence and the presence of polymer crystallinity can affect sorption (Sperling, 1992; Birley *et al.*, 1992). To compensate for this a dual sorption model has been developed, based on Henry's law. This expression states:

$$C^* = C_D + C_H$$

where C^* is the total effective gas concentration, and C_H is the gas concentration assumed to be adsorbed into the holes of the polymer (Birley *et al.*, 1992).

Diffusion is the transport of the gas or vapor molecules through the polymer. This occurs in a direction from high concentration to that of low. This process is expressed by Fick's laws, Fick's first law of steady state transfer states:

$$J = -D (\delta C/\delta x)$$

where J is the flux, the rate of transfer per unit area, D the diffusion coefficient, and $\delta C/\delta x$ is the concentration gradient of the permeant in the x-direction. The diffusion coefficient is very temperature dependent. For unsteady state diffusion Fick's second law states:

$$\delta C/\delta t = D(\delta^2 C/\delta x^2)$$

where the change in rate is proportional to the change in concentration gradient with permeant penetration depth ($\delta^2C/\delta x^2 = 0$ at steady state) (Sperling, 1992; Birley *et al.*, 1992).

There are two basic processes for determining the permeation of the gas or vapor, this is by either the isostatic or quasi-isostatic method. In both cases you have the permeant flowing over one side of the film, which will then permeate through the film and collected. The difference in the two methods is that with the isostatic method the permeant is constantly swept out of a diffusion cell (Figure 1) and carried to a sensor, while with the quasi-isostatic method the permeant is allowed to collect in the diffusion cell and is sampled at certain time periods. Typical profile curves are shown in Figure 2. The test is generally run until steady state is reached.

Protein used alone as the high molecular weight polymer in edible films have generally not displayed good water vapor barrier properties as compared to many synthetic polymers, this is due primarily to their being hydrophilic in nature. Water vapor transmission rate (WVTR) is generally tested using one of two established methods under the ASTM guidelines (ASTM, 1990). The first, ASTM standard E 96-80, is a cup method, while the second, ASTM standard F 1249-90, is a method using an infrared sensor.

In the cup method WVTR is determined by the amount of water weight gained or lost depending if the cup is filled with dessicant or water salt solution respectively. In this method you place a layer of film over a non-corroding water impermeable cup and observe weight change over a period of time. This is continued until a steady rate of weight

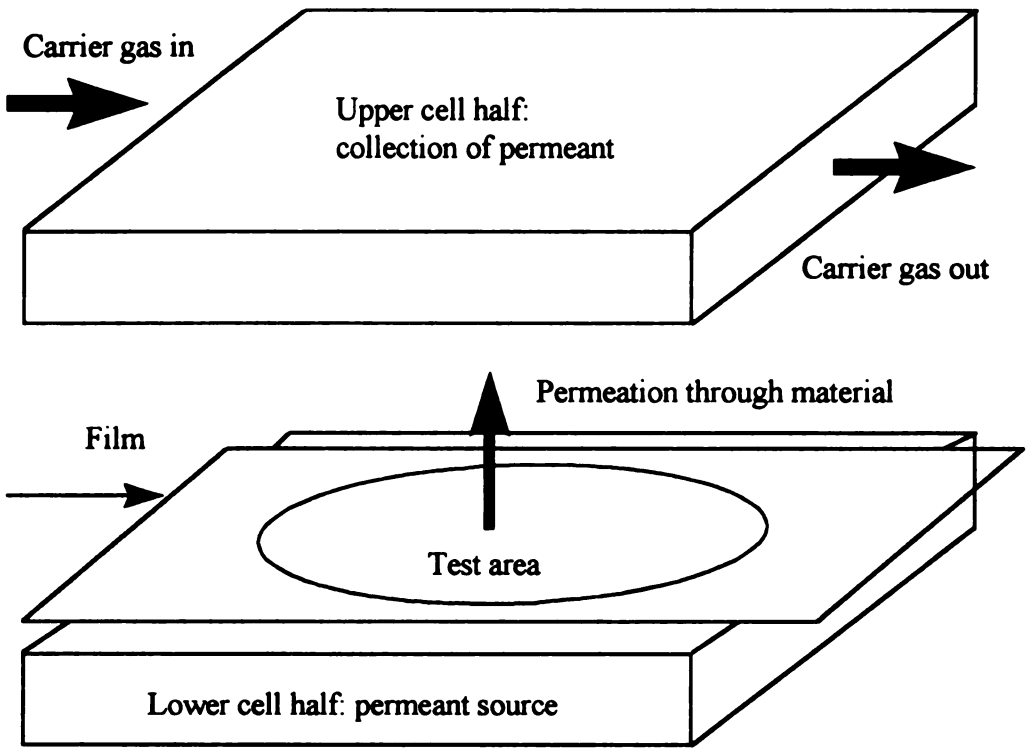
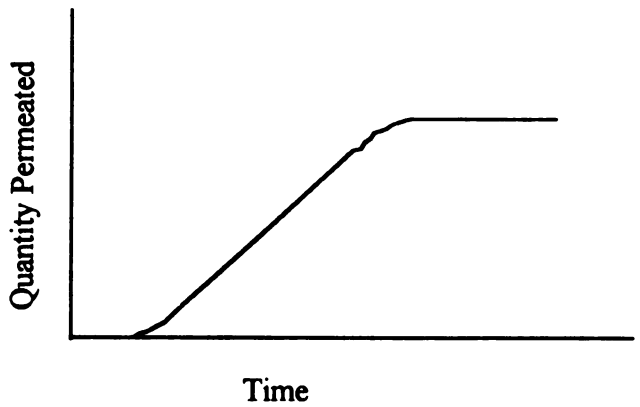


Figure 1. The Diffusion Cell for Permeability Testing

a) Isostatic Method



b) Quasi-Isostatic Method

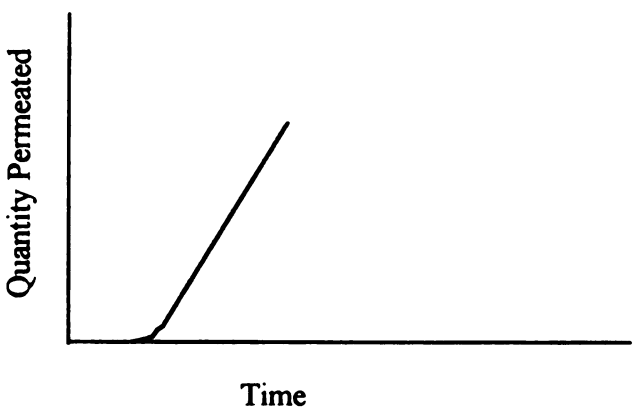


Figure 2. Mass Transport Profile Curves

change is observed. McHugh *et al.*(1993) modified this procedure for use with hydrophilic edible films. This was developed to account for the water vapor partial pressure gradient that is present in the stagnant airspace of the test cup between the salt or water solution and the film. This is expressed by the equation:

$$WVTR = slope / A$$

where slope = slope of line of weight loss vs. Time, A = area of test film.

This is then used to calculate the corrected water vapor partial pressure of the films inner surface in the cup (p_2) by:

$$WVTR = P \times D \times Ln[(P - p_2)/(P - p_1)] / (R \times T \times \Delta z)$$

where P = total pressure, D = diffusivity of water through air at the testing temperature, R = the gas law constant, Δz = mean stagnant air gap height (this should be < 14 mm), p_1 = water vapor partial pressure at the solution surface in the cup. You can then use p_2 to determine the true water vapor permeance by:

$$Permeance = WVTR / (p_2 - p_3)$$

where p_2 = water vapor partial pressure at the films outer surface.

The method using an infrared sensor, ASTM standard F 1249-90, does not take into account weight gain or loss, but rather directly by the amount of a water vapor present in a sample of air. For example, in the isostatic method you would have a carrier gas constantly sweeping out the upper chamber of the diffusion cell, carrying that gas to a sensor which determines the amount of water in it. With the quasi-isostatic method you would inject a certain quantity of an air sample into a sensor which would then determine the amount of water in it.

To obtain the water vapor permeability (WVP) you take into account the WVTR, thickness of the film, and the partial pressure. This is shown by the equation:

$$WVP = (WVTR \times l) / \Delta p$$

where l = thickness, and Δp = partial pressure of water at the test conditions.

Oxygen permeability (OP) is tested in basically the same way that as the infrared method for determining WVP, established under the ASTM standard D 3985-81. First, by obtaining the oxygen transmission rate (OTR) accomplished again by continuously passing a known amount of oxygen containing gas through the lower portion of a diffusion cell and either collecting and sampling, at certain time intervals, the permeant in the upper portion of the diffusion cell, or continuously sweeping out the permeant in the upper portion of the cell, with carrier gas, into a sensor. This is performed until, as in WVTR, steady state is reached. Again, the thickness of the film and the partial pressure is taken into account shown by the equation:

$$OP = (OTR \times l) / \Delta p$$

where l = thickness, and Δp = partial pressure of oxygen (21% O_2 being 1 atmosphere).

Mechanical

The main mechanical properties most commonly tested for in edible films are tensile strength (TS) and elongation (E%). Tensile strength is a measure of the force per unit area required to pull apart the film (F / A), and is an indicator of how strong a film is. Elongation is the length of displacement per original length when a force acts to pull a film apart ($\Delta l / l$) reported as a percentage, and is an indicator of the films toughness and flexibility. Speed at which the force is applied to the sample will affect it's mechanical

properties. The higher the speed at which the force is applied the sample will act more brittle and stiff (Birley *et al.*, 1992). These same results will occur as temperature and R.H. decreases (Birley *et al.*, 1992). The established method for the testing of tensile strength and elongation is ASTM standard D-882-3.

Properties of Protein-Based Films

Water Vapor Permeability

Proteins display hydrophilic tendencies so on their own they have not shown to be very good water vapor barriers. These properties can be affected by different parameters which include protein type and concentration, plasticizer type and concentration, pH of the film and film forming solutions, environmental conditions, and crosslinking agents.

Corn zein films are typically made using ethanol as the solvent. Park and Chinnan (1990) made 83.1% zein protein, 16.9% glycerol films (all film percentages based on dry weight unless otherwise specified) and reported their WVP to range from 7.69-11.49 g mm/m^2 day kPa (tested at 21°C and 85% R.H.). Aydt *et al.* (1991) observed corn zein films made with glycerol as a plasticizer and ethanol as the solvent to display a WVP of 35.15 g mm/m^2 day kPa (tested at 26°C and 100% R.H. inside the test cup and 50% R.H. outside the test cup).

Soy protein films are typically made using distilled water as the solvent. Gennadios *et al.* (1993a) tested the WVP of soy protein isolate (SPI) films as they are effected by pH. They reported that WVP decreased as pH increased above the isoelectric point (pI) of soy protein (pI = 4.5). Stuchell and Krochta (1994) tested the effect of varying amounts of plasticizer (glycerol) on WVP and observed that as plasticizer

concentration increased WVP increased. They also studied the effect of pH change and had the same results as Gennadios *et al.* (1993a).

Wheat gluten films use both ethanol and distilled water as a solvent, because it contains both water and alcohol soluble proteins. Park and Chinnan (1990) tested a wheat gluten film, 75.6% wheat gluten and 24.4% glycerol, and reported it to have a WVP range of 52.1-54.4 g mm/m^2 day kPa (tested at 21°C and 85% R.H.). Aydt *et al.* (1991) observed a WVP of 108.4 g mm/m^2 day kPa (tested at 37.8°C and 100% R.H. inside the cup and 50% R.H. outside) using a film consisting of 71.4% wheat gluten and 28.6% glycerol. It was concluded that wheat gluten films give the lowest WVP's at the extreme pH's, again away from the isoelectric point (pI = 7.5, average for wheat glutens) (Gennadios *et al.*, 1993a). Gontard *et al.* (1992) demonstrated that the amount of ethanol in the film forming solution and it's pH had an important role in WVP. They found that a neutral pH and a low ethanol content gave the lowest WVP's.

Oxygen Permeability

Protein films have generally possessed good oxygen barrier properties as compared to synthetic films. These properties haven't been as widely studied as WVP or mechanical properties. Again, as with WVP factors that can effect these values are protein type, plasticizer, and environmental conditions.

Park and Chinnan (1990) with films comprising of 83.1% corn zein and 16.9% glycerol observed OP's that ranged from 13.0-44.9 cc μm / m^2 day kPa (tested at 30°C and 0% R.H.). Aydt *et al.* (1991) reported an OP of 76.63 cc μm / m^2 day kPa (tested at 37.8°C and 0% R.H.).

Brandenburg *et al.* (1993) with a 62.96% SPI and 37.04% glycerol film observed an OP of 4.75 cc μm / m^2 day kPa (tested at 25°C and 0% R.H.). They also concluded that as pH increased from 6-12 OP decreased.

Gennadios *et al.* (1993b) obtained an OP of 3.82 cc μm / m^2 day kPa with a film consisting of 71.4% wheat gluten and 28.6% glycerol (tested at 23°C and 0% R.H.), and concluded that OP increased as temperature increased. This was verified by Aydt *et al.* (1991) using a film of the same composition, but tested at 37.8°C and 0% R.H., where the OP was observed to be 7.78 cc μm / m^2 day kPa. This was also demonstrated by Rayas (1996) with commercial bread flour, who also showed that the use of crosslinkers (cysteine, formaldehyde, and glutaraldehyde) increased OP, and an increase in pH, from 4 to 11, also increased OP.

Tensile Strength and Elongation

Gennadios *et al.* (1993c) showed that changes conditioning environments (R.H. and temperature) had an effect on tensile strength. They concluded that as R.H. increased, with no change in temperature TS decreased. As temperature increased, with no change in R.H., TS increased. They relate this to the moisture content in the films, with more moisture at higher R.H.'s, acting as a plasticizer, and less moisture present in films at higher temperatures. They observed these film to have a very low E%, ranging from 3-7%.

Gennadios *et al.* (1993a) showed TS for SPI films (62.5% SPI and 37.5% glycerol) to be 3.0-3.6 MPa at a pH of 6-11 and from 1.9-2.3 at a pH in the range of 1-3 MPa. E% peaked for these films between pH 7 and 11, which ranged from 130-

190%. Stuchell and Krochta (1994) showed in SPI films that TS decreased as plasticizer % increased. They found that as plasticizer increased E% increased, films with 20% glycerol had an E% of 16.8%, while films with 23% glycerol had an E% of 23.8%.

Gennadios *et al.* (1993a) observed that as pH of the film increased TS increased, 0.5, 1.9, and 4.4 MPa at pH's of 4, 9, and 13 respectively for films containing 73.2% wheat gluten and 26.8% glycerol. No significant differences were detected in E% at different pH's, ranging from 156.7 to 259.6%. It was demonstrated that as temperature increased, so did TS for wheat protein films. Rayas (1996) showed that the addition of the formaldehyde, a crosslinker, caused and increase in TS, from 2.35 MPa to 4.33 MPa, while cysteine and glutaraldehyde (crosslinkers) had no effect. The addition of the crosslinker decreased the observed E%.

Properties of Milk Protein-Based Films

Water Vapor Permeability

Distilled water is typically used as the solvent for the formation of edible films from milk proteins. Edible films made from non-fat dry milk (NFDM) incorporate all the milk proteins into the film. Maynes and Krochta (1994) produced edible films from various NFDM types. These included commercial blends varying in protein content from 85%-87%, a lactose extracted blend (63.8% protein), and an ultrafiltered (UF) NFDM blend (82.5% protein). The films were consisted of 75% protein mix and 25% glycerol. They observed that the UF-NFDM films gave the best WVP's, at 70.3 g mm/m^2 day kPa, while the rest were not significantly different, ranging from 80.1-86.3 g mm/m^2 day kPa (tested at 30°C with 100% R.H. in the cup, and 0% R.H. outside the cup).

Several groups have studied the properties of whey protein-based edible films. Banjeree and Chen (1995) compared properties of whey protein concentrate (WPC), 76.6% protein, and whey protein isolate (WPI), 93.6% protein. The films consisted of 66.7% protein and 33.3% glycerol. They reported that the WPC films gave a lower WVP then WPI, 10.64 g mm/m^2 day kPa and 12.12 g mm/m^2 day kPa respectively (tested at 23°C with 100% R.H. in the cup, and 55% R.H. outside the cup). McHugh *et al.* (1994a) concluded that both plasticizer type and plasticizer concentration effect the WVP of a film. They demonstrated that sorbitol, as a plasticizer, displayed better water barrier properties than either polyethylene glycol (PEG) or glycerol at the same concentration, with glycerol having the worst barrier properties. Values of 50% WPI, 50% plasticizer being 3.53, 5.61, and 6.44 g mm/m^2 day kPa for sorbitol, PEG, and glycerol respectively (tested at 25°C and ≈ 77% R.H. inside the cup and 0% outside). They also showed that as the concentration of plasticizer increased WVP of the films increased. Films with 62.5% protein an 37.5% sorbitol had a WVP of 2.58 g mm/m^2 day kPa, while films with 50% WPI and 50% sorbitol had a WVP of 3.53 g mm/m^2 day kPa.

Various caseinates have been studied in the production of films (including sodium, calcium, potassium, and magnesium caseinates). Banjeree and Chen (1995) reported that calcium caseinate (CC) films gave a lower WVP than sodium caseinate (SC) and potassium caseinate (PC) films, values being 7.91, 12.90, and 12.12 g mm/m^2 day kPa respectively (tested at 23°C and 100% R.H. inside the cup and 55% outside). Avena-Bustillos and Krochta (1993a) also obtained the same results, with CC films providing a better water barrier than SC films. They also tested the effect of calcium crosslinking and pH adjustment on WVP properties, and showed that soaking SC films in a calcium

chloride solution (calcium crosslinker) or in buffers to lower pH to 4.6 (pI of casein) decreased the WVP of the film. Ho (1992) produced films out of 80% magnesium caseinate and 20% glycerol, and 80% rennet casein and 20% glycerol, and obtained WVP's of 43.9 and 56.0 g mm/m^2 day kPa respectively (tested at 25°C and a corrected R.H. of 77%).

Oxygen Permeability

McHugh and Krochta (1994b) studied the effect of plasticizer type, plasticizer concentration, and relative humidity on OP of WPI-based films. They concluded that films containing sorbitol will give lower OP's than films containing glycerol, at equal concentrations. At 70% WPI and 30% plasticizer the OP of the film using sorbitol had an OP of 4.3 cc μm / m^2 day kPa, compared to 76.1 cc μm / m^2 day kPa for glycerol (tested at 23°C and 50% R.H.). They demonstrated that as plasticizer concentration increased OP increased, and as relative humidity increased OP increased.

Tensile Strength and Elongation

Maynes and Krochta (1994) observed TS's up to 9.1 MPa with 75% protein and 25% glycerol NFDM films. UF-NFDM films displayed the lowest E% at 5.2%, the lactose extracted NFDM film had an E% of 12.2%, while the commercial brands ranged from 22.1-38.5%, with E% increasing as protein content decreased. McHugh and Krochta (1994b) reported TS increased as plasticizer concentration decreased in WPI and glycerol films. 85% WPI and 15% glycerol films had a TS of 29.1 MPa, while 70% WPI and 30% glycerol films had a TS of 13.9 MPa. As plasticizer concentration increased E%

increased. Banjeree and Chen (1995) demonstrated that WPI films produced stronger films than WPC, with TS's of 5.94 and 3.36 MPa respectively (66.7% protein and 33.3% glycerol). E% for the WPC film was 20.84% and 22.74% for the WPI film. Banerjee and Chen (1995) observed that CC films produced stronger films than SC or PC, TS's of 4.25, 2.98, and 2.97 MPa respectively. The PC film had the largest E% followed by SC and CC, elongations being 42.80, 29.89, and 1.45%, respectively. Motoki *et al.* (1987) used transglutaminase as a crosslinker, which increased TS of α_{s1}-casein films from 4.1 to 10.6 MPa.

Milk protein-based edible film and coating applications

The ultimate goal for developing these protein edible films is for their possible application into a food system. There has been numerous studies using these protein solutions as a coating on either meat, seafood, nuts, fruits, and vegetables (Baker *et al.*, 1994). These studies have shown the ability to reduce spoilage problems, like degradation from oxidative rancidity and browning, in these foods. These coatings are either brushed on, sprayed on, or the food item is dipped into the coating.

Stuchell and Krochta (1995) used a WPI and acetylated monoglyceride coating on frozen king salmon and found it to delay lipid oxidation, decrease peak peroxide value, and reduce the amount of moisture loss. Lerdthanangkul and Krochta (1996) observed that SC coatings caused an increase in internal CO_2 and a decrease in internal O_2 in green bell peppers, showing their good gas barrier properties. Avena-Bustillos *et al.* (1993b) showed that SC-lipid and CC-lipid coatings reduced white blush on minimally processed carrots, and also reduced water vapor transmission. Most of this research dealt with

composite (protein/lipid) coatings, because the protein coatings on their own do not provide good water barrier properties. There has been little reported research with free-standing protein edible films, this is due largely to the fact that a way to seal these films has not yet been reported.

Properties of Plasticizers

Glycerol and sorbitol are naturally occurring carbon backboned polyhydric alcohols. Besides use as plasticizers they are often used as sweeteners, humectants, and pharmaceutic aids.

Glycerol originates from oils and fats, usually as a by-product in the manufacturing of soaps and fatty acids. It has a molecular weight of 92.09 daltons and is a three carbon molecule with one hydroxyl group ($C_3H_8O_3$) (Merck, 1989). It is a liquid at room temperature, possessing a melting point of 17.8°C. It is miscible in water and alcohol and have the ability to absorb moisture from the air. It's viscosity at 20°C is 1.143, 2.095, 6.050, and 22.94 centipoise for solutions of 5, 25, 50, and 70% glycerol respectively (Merck, 1989).

Sorbitol was discovered in 1872 in the berries of mountain ash (*Sorbus aucuparia L.*) and now are produced by high pressure hydrogenation or electrolytic reduction of $_D$-glucose, or by catalytic hydrogenation of dextrose (Merck, 1989). It has a molecular weight of 182.17 daltons, consisting of a 6 carbon chain with 4 hydroxyl groups ($C_6H_{14}O_6$) (Merck, 1989). It is mainly found in the stable crystalline (γ-) form, with a melting point of 96°C. It is highly soluble in water (solubility at 25°C being 234g/100g water), but insoluble in most other organic substances (Sicard and Leroy, 1983; Sicard,

1982). It is stable and chemically unreactive. It is more viscous than lower weight polyhydric alcohols, with viscosities at 20°C being 1.230, 2.689, 11.09, and 185 centipoise for solutions of 5, 25,50, and 70% sorbitol respectively (Merck, 1989). At equal R.H.'s the water content of sorbitol will be lower than that of glycerol. Sorbitol is also more resistant to changes in water content as relative humidity changes (Sicard and Leroy, 1983).

Properties of Milk Proteins

Milk is comprised of 3.3-3.9% protein, of which 20% are whey proteins and 80% are casein proteins (Swaisgood, 1985). Caseins possess many desirable characteristics that make them suitable for the production of edible films.

Casein

On average, 38-45% of the caseins are comprised of α_{s1}-casein (Leman and Kinsella, 1989; Swaisgood, 1985; Dalgleish, 1982). This protein has been determined to be 199 fatty acid residues long, with a calculated molecular weight of about 23,000 daltons, depending on the variant (Swaisgood, 1985; Dalgleish, 1982). The C-terminal is comprised of some α–helices, β-sheets, β-turns, and unordered structure, while the N-terminal is mainly random coil in structure (Swaisgood, 1985). The secondary structure is limited due to the presence of proline, making up about 8.5% of the residues (Kinsella, 1984).

There are two variants of this protein, with the difference being in the number of phosphoseryl groups, either 8 or 9 (Swaisgood, 1985; Dalgleish, 1982; Fox and Mulvihill,

1982). The variant with 9 phosphoseryl groups is referred to as α_{s0}-casein. Even though threonine, which is present, is capable of phospohorylation, this occurrence is uncommon (Dalgleish, 1982). These phosphoseryl groups allow the protein to bind Ca^{2+}. α_{s1}-casein can bind up to 8-10 moles Ca^{2+}/mole of protein under normal circumstances and up to 20 mole Ca^{2+}/mole protein at high Ca^{2+} concentrations (Swaisgood, 1985; Dalgleish, 1982; Fox and Mulvihill, 1982). In both variants all but one of the phosphoseryl groups are found between residues 41 and 80. Three hydrophobic regions are present in α_{s1}-casein, from residues 1-40, 90-110, and 130-199 (Fox and Mulvihill, 1982). The overall charge being -21mV at pH 6.8, with a hydrophobicity of 1172 cal/residue (Kinsella, 1984; Dalgleish, 1982).

The α_{s2}-caseins comprise 10-12% of the casein proteins (Leman and Kinsella, 1989; Swaisgood, 1985; Dalgleish, 1982). It consists of a 207 amino acid residue chain, with a calculated molecular weight of 23,000-25,000 daltons, depending on the variant (Swaisgood, 1985; Kinsella, 1984; Dalgleish, 1982). Due again to the presence of proline, about the same amount as α_{s1}-casein, any secondary structure is limited (Kinsella, 1984; Dalgleish, 1982).

There are 4 variants of α_{s2}-casein, again differing in the number of phosphoseryl groups, ranging from 10-13, termed as α_{s6}-casein, α_{s4}-casein, α_{s3}-casein, and α_{s2}-casein respectively (Kinsella, 1984; Dalgleish, 1982; Fox and Mulvihill, 1982). These phosphoseryl groups are fairly evenly distributed throughout the protein. However, the C-terminal, residues 160-207, is hydrophobic. There are two cysteine residues in the protein, which could participate in disulfide bonds. It is the most hydrophillic of the casein proteins, with a hydrophobicity of 1111 cal/residue, and thus the most ionic strength

dependent. The overall charge ranges from -16 to -22mV at pH 6.8, depending on the variant (Kinsella, 1984; Dalgleish, 1982).

β-casein makes up 31.3-36% of the casein proteins (Leman and Kinsella, 1989; Swaisgood, 1985; Dalgleish, 1982). This protein is 209 residues in length, with a molecular weight of 23,900-23,980 daltons (Swaisgood, 1985; Kinsella, 1984; Dalgleish, 1982). β-casein is comprised of 10% α-helix, 13% β-sheet, and 77% unordered structure (Andrews *et al.*, 1979). This random structure is again the result of the presence of 16% proline residues (Kinsella, 1984). There are five phosphoseryl groups which are all located in the N-terminal. It can bind 4-5 mole Ca^{2+}/mole of protein (Swaisgood, 1985). β-casein is the most hydrophobic of all the casein proteins, with an overall hydrophobicity of 1334 cal/residue, but the hydrophobic C-terminal, residues 30-209, has a hydrophobicity of 1408 cal/residue, and carries a net charge of -12mV at pH 6.8 (Kinsella, 1984; Dalgleish, 1982). It is the most temperature dependent of the caseins (Swaisgood, 1985).

There is a class of casein derived from the proteolysis of β-casein. These are referred to as γ-casein, and represent 3-5% of the total casein fraction (Swaisgood, 1985; Fox and Mulvihill, 1982). The variants of this protein γ_1-casein, γ_2-casein, and γ_3-casein have chain lengths of 181, 104, and 102 residues respectively, with molecular weights of 20,520, 11,822, and 11,557 Daltons (Swaisgood, 1985).

β-casein can be hydrolysed at 1 of 3 lysyl residues, 28, 104, and 106, forming 1 of three pairs of polypeptide chains, of which one half of each pair is lost into the serum, with the other half making up the γ-caseins (Fox and Mulvihill, 1982). Of these γ_1-casein is the only one containing a phosphoseryl group.

The final casein protein is κ-casein, which is found at levels of 10-13% (Leman and Kinsella, 1989; Swaisgood, 1985; Dalgleish, 1982). It contains 169 fatty acid residues, with a molecular weight of 19,000 daltons (Swaisgood, 1985; Kinsella, 1984; Dalgleish, 1982). Loucheaux-Lefebvre *et al.* (1978) determined that κ-casein consisted of 26% α-helix, 31% β-sheet, and 24% β-turns. They determined that residues 105-106 probably formed either an α-helix or β-sheet, between two stable β-turns and another β-turn at residues 113-116, making that linkage accessible to proteolysis. There is only one phosphoseryl group and two cysteine residues (Swaisgood, 1985; Dalgleish, 1982). κ-casein can contain from 0-3 oligosaccharide chains. The carbohydrate moiety exists as either a tri- or tetrasaccharide, composed of *N*-acetyl-neuraminic acid, galactose, and *N*-acetylgalatosamine. The main point of attachment is at threonine 133, with attachment occurring at threonine 131 and threonine 135 (Fox and Mulvihill, 1982). Due to the presence of only 1 phosphoseryl group κ-casein can only bind 1-2 mole Ca^{2+}/mole protein at pH 6.8 (Swaisgood, 1985). The overall hydrophobicity is 1224 cal/residue, with an overall charge of -4 mV (Swaisgood, 1985; Dalgleish, 1982). Due to the structure of κ-casein the bond between the phenylalanine (105) and methionine (106) residues is susceptible to proteolysis by rennet (or chymosin). This gives rise to the hydrophobic N-terminal portion, known as para-κ-casein, with a hydrophobicity of 1310 cal/residue and a charge of +5 mV, and the more hydrophilic macropeptide containing the oligosaccharides, with a hydrophobicity of 1082 cal/residue, carrying a charge of -8 mV (Dalgleish, 1982). The macropeptide at this occurrence is then lost into the serum.

The casein micelle

The casein proteins along with colloidal calcium phosphate (CCP) interact with each other to form spherical complexes known as micelles. The size of the micelles range from 10-300 nm, with a molecular weight of 10^8 to 10^9 daltons (Swaisgood, 1985; Kinsella, 1984; Dalgleish, 1982). The composition of the micelle is 92-94% casein protein and 6-8% CCP (Swaisgood, 1985; Fox and Mulvihill, 1982). The structure of the micelle is based on either of two generally accepted theories, both involving the concept of submicelles, 10-20 nm in size, aggregating to form the micelle (Swaisgood, 1985; Kinsella, 1984; Fox and Mulvihill, 1982).

The first casein micelle model was presented by Slattery and Evard (1973) and updated (1979). In this model the submicelle is formed containing a partially hydrophobic, α_s- and β-caseins, portion and a partially hydrophilic portion, κ-casein. It is then probable that a tetrahedral arrangement is formed, which will keep growing until there is enough κ-casein on the surface to prevent any further hydrophobic interactions (Figure 3). The micelle is stabilized by the collective presence of the hydrophobic interactions of α_{s1}- and β-caseins, and through the formation of calcium phosphate salt bridges within the interior (Slattery, 1976).

The second model was presented by Schmidt (1980). His model also uses the submicelle theory stemming from the interaction of α_{s1}- and κ-casein, the self-associations of α_{s1}- and β–casein by hydrophobic bonding, and also through the self-association of α_{s2}-casein by electrostatic interactions. The submicelles are spherical particles with a hydrophobic core and a surface layer with the phosphate groups of α_{s1}-, α_{s2}-, and β-casein and the polar macropeptide portion of κ-casein. Micelle formation begins with the

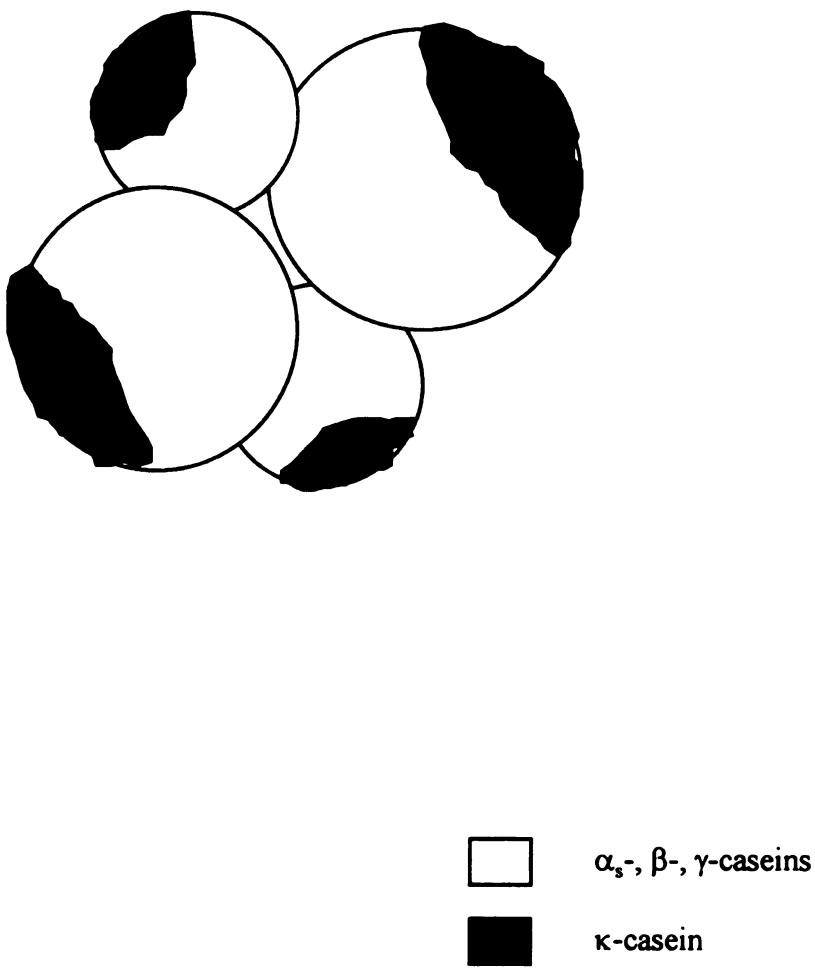


Figure 3. The Casein Micelle (Slattery and Evard Model)

aggregation of tertiary colloidal calcium phosphate and the submicelles. Growth will continue until, as in Slattery's model, the surface is mainly κ-casein. Submicelles deficient in κ-casein will be located in the core.

The CCP consists of ions of calcium, phosphate, and some magnesium and citrate, comprising an average of 2.8-2.9, 4.3-5.2, 0.1, and 0.4-0.5% of the total micelle respectively (Brunner, 1977; Schmidt, 1980). It is found in an apatite-like complex of tertiary calcium phosphate, with some calcium citrate (Schmidt, 1980). Magnesium prevents the calcium phosphate from transforming into a more stable hydroxyapatite form, while the casein prevents flocculation (Schmidt, 1980; Swaisgood, 1985). Calcium acts to neutralize some of the repulsive electrostatic (-) charges and facilitates hydrophobic interactions. Calcium also plays a compacting role in the micelle, through the salt bridges.

The casein micelle is rather porous and thus is highly hydrated, containing from 2-3.7 grams H_2O/gram dry protein (Swaisgood, 1985; Kinsella, 1984; and Fox and Mulvihill, 1982). The presence of some κ-casein in the interior, as much as 30% of the κ-casein in the micelle, probably allows the hydrophobic interior to stay stable (Slattery, 1976).

Casein manufacturing

The manufacturing of casein for use as a food ingredient is accomplished by one of two ways, lactic acid or rennet precipitation.

In lactic acid casein, pastuerized skim milk is inoculated with the lactic starter culture and allowed to incubate for 14-16 hours at 22-26°C. The fermentation of lactose causes the pH to reduce to about 4.6, causing the casein to coagulate and form a curd.

With rennet casein, calf rennet or chymosin is added to pasteurized skim milk at 29°C. This enzyme cleaves the κ-casein, causing it to lose the macropeptide into the serum and destabilizing the micelle, bringing on the clotting of the casein. This process takes about 30 minutes under a pH of 6.6 (Southward and Walker, 1980).

The remaining processing required is the same for both types of precipitated caseins. First, the curd is cooked at 50-55°C to firm it up to withstand the rest of the processing (Muller, 1982). The curd and whey then go through several separation and washing stages. The casein is then dewatered, dried, milled, sieved, blended, and packaged (Muller, 1982; Southward and Walker, 1980).

Properties of these caseins include insolubility at pH 7.0 and 4.6-4.7 for rennet casein and lactic acid casein respectively. Acid caseins are able to be solubilized at pH 4.6-4.7 with the use of alkalis or alkaline salts, these are referred to as caseinates (Kirkpatrick and Walker, 1985). The common alkalis and alkaline salts used are calcium, sodium, and potassium. Both types of precipitated casein are heat stable and have good nutritive qualities.

MATERIALS AND METHODS

Film Components and Formation

Casein samples were obtained from New Zealand Milk Products (N. America) Inc., (Santa Rosa, CA). Lactic acid casein being Alacid 710, 30 mesh and rennet casein being Alaren 771, 30 mesh (Table 1). Protein and ash content of the casein samples were verified by standard methods (AOAC, 1990). Sorbitol was purchased from Sigma Chemical Co., (St. Louis, MO), and glycerol was purchased from Mallinckrodt Specialty Chemicals Co., (Paris, KY).

Figure 4 shows a schematic diagram of the film forming process. The various casein based edible films were prepared by first mixing lactic acid or rennet casein (3,5, or 7% w/w) with sorbitol or glycerol (5% w/w), distilled water, and 1M NaOH (to adjust pH to 10.0) for final mixtures of 150 g (Table 2 and Table 3). These were then heated and stirred, using the "Magna-4" magnetic stirrer and hot plate, model 4820-4 from Cole-Parmer (Chicago, IL), to a final temperature of 65.5 ± 2.5°C (150°F) for 30 minutes, and then held at that temperature for 15 minutes. The final pH of the film forming solution was measured using the Corning pH meter 240 (Corning, NY). These were the conditions upon which the best solubility of the caseins were obtained.

Next, samples were filtered twice, through 1 layer of cheesecloth. Then they were stored at 20 ± 2.0°C (68°F) for 4 hours to allow any foaming created during the mixing process to settle. A vacuum was applied to solutions for 30 minutes, using a hydrometric vacuum system, to remove any residual air in the solution. The film forming solution was

Table 1. Compositions of Casein Protein Powders Used[1]

Percent (%)	Lactic Acid Casein Alacid 710	Rennet Casein Alaren 771
Protein (N x 6.38) %	87.3	80.6
Ash %	1.8	7.8
Moisture %	9.6	11.0
Fat %	1.2	0.5
Lactose %	0.1	0.1
pH (5% at 20°C)	4.6	7.1

[1] Values based on company specifications (New Zealand Milk Products (N. America) Inc.)

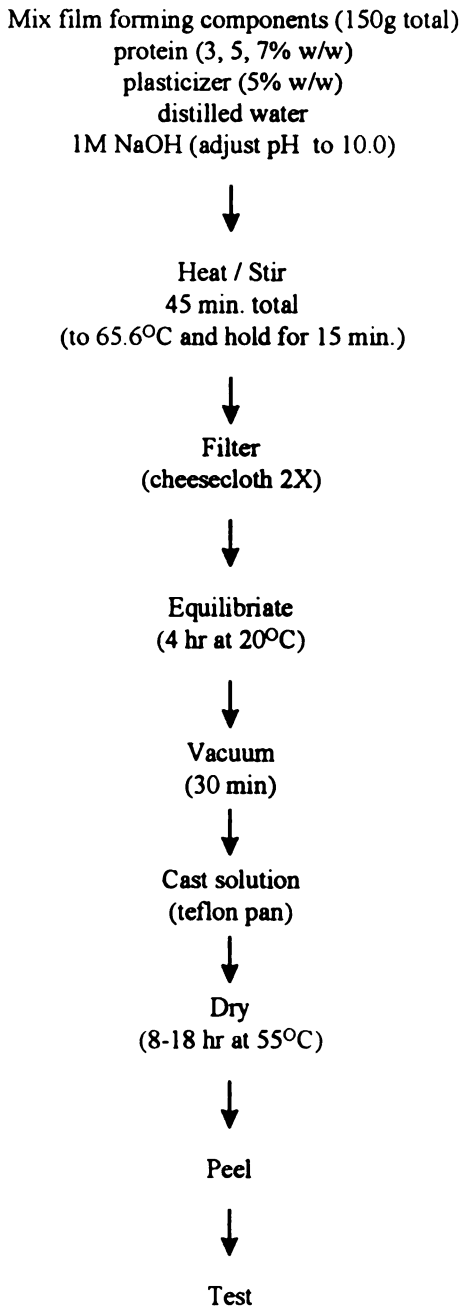


Figure 4. Schematic Diagram of the Film Forming Process

Table 2. Compositions of Casein-Based Edible Films

Protein, Plasticizer Type[1]	%protein powder/ %plasticizer wet weight	%protein powder/ %plasticizer dry basis
L.A. Casein, S	3.0/5.0	37.5/62.5
L.A. Casein, S	5.0/5.0	50/50
L.A. Casein, S	7.0/5.0	58.3/41.7
L.A. Casein, S	3.0/5.0	37.5/62.5
L.A. Casein, S	5.0/5.0	50/50
L.A. Casein, S	7.0/5.0	58.3/41.7
R. Casein, G	3.0/5.0	37.5/62.5
R. Casein, G	5.0/5.0	50/50
R. Casein, G	7.0/5.0	58.3/41.7
R. Casein, G	3.0/5.0	37.5/62.5
R. Casein, G	5.0/5.0	50/50
R. Casein, G	7.0/5.0	58.3/41.7

[1] L.A. Casein=Lactic acid casein, R. Casein=Rennet casein; S=Sorbitol, G=Glycerol.

Table 3. Formulations of Film Forming Solutions for Casein-Based Edible Films

Casein Type (% w/w)	Protein Powder (g)	Plasticizer (5% w/w) (g)	Distilled H_2O (g)	NaOH (g)
Lactic Acid				
low (3%)	4.5	7.5	133.2	4.8
medium (5%)	7.5	7.5	127.0	8.0
high (7%)	10.5	7.5	120.7	11.3
Rennet				
low (3%)	4.5	7.5	136.8	1.2
medium (5%)	7.4	7.5	132.9	2.1
high (7%)	10.5	7.5	129.1	2.9

then cast on a 7.5 in diameter teflon coated pan. A teflon coated pan was chosen because upon drying on glass, films were unable to be peeled. The amount of film forming Solution cast varied depending on the protein content, 52.5 ± 2.5 ml of the medium and high protein content films and 90 ± 5 ml of the low protein content films. These casting volumes were used to obtain dried films with an average thickness of 0.203 mm (8 mils). Films were dried in a gravity convection incubator, Blue M Electric Co., (Blue Island, IL), at 55 ± 2°C (130°C) until they were able to be peeled from the casting surface. The greater amount of solution cast the longer the drying time was, because of the increased solvent amount. Drying times were about 8 hours for the high and medium protein content solutions and about 18 hours for the low protein content solutions. Once peeled from the casting surface, films were kept at 20 ± 2°C (68°F) until testing was performed.

Thickness

Thickness measurements was measured using a micrometer, TMI model 549M micrometer from Testing Machines Inc,. (Amittyville, NY). For barrier testing, thickness was the average of 5 measurements, for mechanical testing it was the average of 3. Barrier testing was done on film samples of 0.203 ± 0.038 mm (8.0 mils), while thickness for mechanical testing was 0.203 ± 0.089 mm (8.0 mils).

Water Vapor Permeability

WVTR was tested according to ASTM standard F 1249-90, "Water vapor transmission rate through plastic film and sheeting using a modulated infrared sensor." They were tested using the Permatran-W (MoCon Inc., Minneapolis, MN). The samples

were tested at 37.8 ± 0.5°C (100°F) and 90 ± 3% R.H. A saturated salt solution of potassium nitrate ($NH_4H_2PO_4$) was used to obtain the desired R.H.

Samples which were placed in a 50 cm^2 diffusion cell with the absorbent pad in the bottom half of the cell soaked with the salt solution, with dry air sweeping out the top half of the cell going to the sensor (Figure 1). The testing surface area of the casein film samples were reduced from 50 cm^2 to 5 cm^2 with the use of a foil backing. Otherwise, they would adsorb all the water from the salt solution. Samples were conditioned for 10 hours at the testing conditions before testing was conducted. A calibration sample, with a known WVTR , was run with all test samples. The calibration sample was 1 mil thick Mylar, with a WVTR of 21 g H_2O/ $m^2 \cdot$ day. Tests were run until steady state was reached at which point 12 readings were taken over a 30 minute period. These readings (in mV) were then averaged then adjusted according to the calibration sample to get WVTR. WVP was then calculated by the equation:

$$WVP = WVTR \times l / \Delta p$$

l = thickness of film

Δp = partial pressure of water at test conditions

WVP values were the average of samples done in triplicate.

Oxygen Permeability

OTR was measured according to ASTM standard D-3985-81, "Oxygen gas transmission rate through plastic film and sheeting using a coulometric sensor." Tests were run at 23 ± 2°C, 0 % R.H., and 21% oxygen, using the Oxtran 200 (MoCon Inc., Minneapolis, MN). All samples were conditioned for 10 hours at the same conditions

prior to testing. Temperature was maintained using a water bath system, Endocal water bath (Neslab Instruments Inc., Newington, NH).

The testing area of the sample was 50 cm^2, with compressed air at 21% oxygen sweeping the bottom half of the cell and nitrogen sweeping the top half, going to the sensor. OP was calculated by the equation:

$$OP = OTR \times l / \Delta p$$

l = thickness of film

Δp = partial pressure of oxygen

OP values were the average of samples done in triplicate.

Mechanical Properties

TS and E were tested according to ASTM standard D-882-83, "Tensile properties of thin plastic sheeting." Tests were run using the Instron Universal Tester, model 2401 from Instron (Canton, MA), with a 1kN static load cell and crosshead speed of 20 in./min. Conditions of testing were 23 ± 2°C (73.4°F) and 50 ± 5% R.H. All samples were conditioned for 48 hours at the same conditions prior to testing (Banerjee and Chen, 1995). Testing sample size was 2 in. x 1 in. TS was determined by the equation:

$$TS = load / area$$

load = peak force

area = sample width x sample thickness

E was determined by the equation:

$$E = \Delta l / l \text{ (expressed as a percentage)}$$

Δl = distance sample stretched

l = original length of sample

TS and E values are the average of triplicate samples. Each sample was tested in duplicate.

Storage Study

Two representative treatments of the casein-based edible films developed in this research were further tested in a storage study using American processed cheese slices to investigate their effectiveness as a packaging wrap. Casein-based edible films used for this study were the 50% lactic acid casein/50% sorbitol and 58.3% lactic acid casein/41.7% glycerol films (percentages on a dry basis). These films were chose due to their similar barrier and mechanical properties they possessed, which were best the overall among the treatments evaluated in this research. Thickness of the films varied from 5.19 to 8.01 mils (0.131 to 0.203 mm). Slices unwrapped and in the original LDPE wrapper were used as controls. The processed American cheese slices used for this storage study, were purchased at a local retail outlet (East Lansing, MI). Cheese slices (3.25 in. x 3.5 in.) were placed between two layers of casein based film, sealed together by rubber cement, then dipped in paraffin wax to minimize water loss through the seal (Figure 5). The cheese samples were stored at 2.2 ± 1.0°C (36°F) and 88 ± 5% R.H. Triplicate samples of each treatment were tested every 5 days over a 30 day storage period. Both the cheese and the wrap were tested for color change and moisture content during storage.

Color tests were performed using the HunterLab colorimeter from (Hunter Associates Laboratory, Inc., Reston, VA), using a black and a white standard tile for

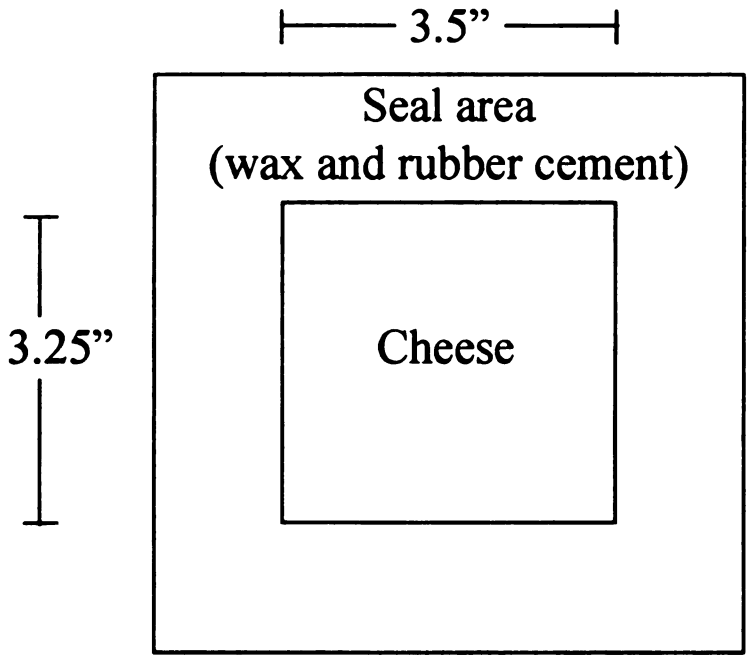


Figure 5. Packaging of Processed Cheese Slices Using Casein-Based Edible Films.

calibration, and the black tile as the background when testing samples. Values of L (black to white), a (green to red), and b (blue to yellow) were determined. Moisture content of the cheese was performed according to the "Standard Methods for the Examination of Dairy Products," (Marshall 1992). Cheese was shredded and approximately 3.0 ± 0.5 g was placed in an aluminum weighing dish and dried at 80 ± 3°C (176°F) using a gravity convection oven from Precision Instruments (Chicago, IL), until a constant weight was reached (approximately 16 hours). After the samples were dry they were placed in a dessicator for 30 minutes to cool and reweighed. Moisture content (%Moisture) was calculated by the following equation:

%Moisture = [(wt. initial - wt. final)/wt. initial] x 100

Moisture content of the films were determined by drying 3.0 ± 0.5 g of the edible film for 16 hours at 100 ± 3°C (212°F), moisture of the samples were calculated similar to the cheese.

Statistical Analysis

Statistical analysis of the effect of protein type, plasticizer type, protein to plasticizer concentration on film properties, and the effect of wrap type on moisture content and color of processed cheese and their wraps during storage were made using Sigma Stat 1.0 from the Jandel Corp., (San Rafael, CA) performing multiple comparisons with the Student-Newman-Keuls method.

RESULTS AND DISCUSSION

Film Development

Verification analysis of the protein powders showed that the specified values in Table 1 were within reason, protein content upon analysis being 90.18% and 83.4% for the lactic casein powder and rennet casein powder respectively, and ash being 1.28% and 8.02% respectively.

Upon drying all films were smooth, flexible, and transparent. However, films containing low protein content, 37.5% protein and 62.5% plasticizer (all percentages in results and discussion are on a dry basis unless otherwise specified), displayed a tackiness that the high and medium protein content films did not. Films made with a plasticizer content higher than 62.5% were too tacky and fell apart upon peeling from the casting surface, thus were not used in this study. The film solution made with rennet casein and glycerol, 58.3% protein and 41.7% plasticizer, gelled shortly after the heating process, thus it had to be cast immediately following heating and filtering. Films made with any higher protein content were too brittle and could not be peeled from the casting surface.

Barrier Properties

Water vapor permeability (WVP)

A WVP of 34.0 g·mm/day·m^2·kPa was observed with lactic acid casein and sorbitol, 58.3% protein and 41.7% plasticizer, films. This was the lowest WVP observed in this study. Films made with lactic acid casein and glycerol, 50% protein and 50% plasticizer, displayed a WVP of 59.3 g·mm/day·m^2·kPa, which was the highest observed

(Table 4, 5; Figure 6). In general, films made with sorbitol displayed lower WVP's than films made with glycerol, at same protein plasticizer ratios, with a wider difference among the lactic acid casein films. A significant difference ($p<0.05$) was witnessed among the high protein, lactic acid casein films, 34.0 g·mm/day·m^2·kPa and 54.7 g·mm/day·m^2·kPa for films containing sorbitol and glycerol respectively (Table 4). Protein type did not play a significant role in the WVP of the films (Table 5). No trends were found pertaining to protein concentration.

It had been shown in other studies using sorbitol and glycerol as the plasticizer that films made with sorbitol displayed lower WVP's then those made with glycerol (McHugh and Krochta 1994). This is due to the ability of glycerol to adsorb water more than sorbitol, probably stemming from the more crystalline structure of sorbitol, making it more stable (Sicard and Leroy, 1983). It was thought that as protein content increased in the films a significant decrease in WVP would occur, due to more protein-protein interactions in the films matrix. Even though this trend did occur, however not statistically significantly, the high plasticizer content in the films was probably high enough to counteract the significance of these interactions in preventing the passage of water vapor. WVP's didn't vary between the two protein types, even though protein content in the powder was substantially higher with the lactic acid casein (87.3% to 80.6% for rennet casein). This was probably counteracted by higher portion of fat, being hydrophobic, and ash, containing calcium that can promote crosslinking of the proteins.

Table 4. Effect of Plasticizer Type on Water Vapor Permeability (WVP) of Casein-Based Edible Films (37.8°C, 90% R.H.)

Treatment[1] (Protein Powder%/ Plasticizer%)	Lactic Acid Casein[2,3]	Rennet Casein[2,3]
S(37.5/62.5)	44.9 ± 9.8^{ab}	49.7 ± 8.3^{ab}
S(50/50)	45.0 ± 9.0^{ab}	49.6 ± 6.6^{ab}
S(58.3/41.7)	34.0 ± 5.2^{b}	39.6 ± 3.6^{b}
G(37.5/62.5)	54.9 ± 1.6^{a}	57.9 ± 4.9^{a}
G(50/50)	59.3 ± 6.5^{a}	58.2 ± 2.5^{a}
G(58.3/41.7)	54.7 ± 6.2^{a}	45.2 ± 6.8^{ab}

[1] Letter denotes plasticizer type: L=Lactic acid casein, R=Rennet casein; Protein powder%/plasticizer% is reported as a dry basis.

[2] Different letters columnwise denote significant difference ($p<0.05$).

[3] Mean ± s.d. are reported as g · mm/day · m^2 · kPa.

Table 5. Effect of Casein Type on Water Vapor Permeability (WVP) of Casein-Based Edible Films (37.8°C, 90% R.H.)

Treatment[1] (Protein Powder%/ Plasticizer%)	Sorbitol[2,3]	Glycerol[2,3]
L(37.5/62.5)	44.9 ± 9.8^a	54.9 ± 1.6^a
L(50/50)	45.0 ± 9.0^a	59.3 ± 6.5^a
L(58.3/41.7)	34.0 ± 5.2^a	54.7 ± 6.2^a
R(37.5/62.5)	49.7 ± 8.3^a	57.9 ± 4.9^a
R(50/50)	49.6 ± 6.6^a	58.2 ± 2.5^a
R(58.3/41.7)	39.6 ± 3.6^a	45.2 ± 6.8^a

[1] Letter denotes casein type: L=Lactic acid casein, R=Rennet casein; Protein powder%/plasticizer% is reported as a dry basis.

[2] Different letters columnwise denote significant difference ($p<0.05$).

[3] Mean ± s.d. are reported as $g \cdot mm/day \cdot m^2 \cdot kPa$.

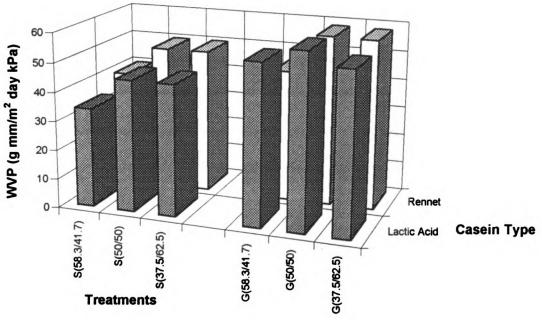


Figure 6. Water Vapor Permeability (WVP) of Casein-Based Edible Films (37.8°C, 90% R.H.)
Treatment: S=Sorbitol, G=Glycerol;
(Protein Powder%/Plasticizer%)

Oxygen permeability (OP)

Films made with lactic acid casein and sorbitol, 37.5% protein and 62.5% plasticizer, gave the lowest OP of 0.653 cc·μm/day·m^2·kPa. Films made with rennet casein and glycerol, 37.5% protein and 62.5% plasticizer, gave an OP of 7.057 cc·μm/day·m^2·kPa, which was the highest OP observed (Table 6, 7; Figure 7). Plasticizer type had an effect on OP only when rennet casein was the protein used (at low and medium concentrations) and when glycerol was the plasticizer. These values were significantly higher ($p<0.05$) than the rest (Table 6). Films made with lactic acid casein and glycerol, 50% protein and 50% plasticizer, displayed a significantly higher ($p<0.05$) OP than the rest of the lactic acid casein films. Protein type had a significant effect ($p<0.05$) on OP when glycerol was used as the plasticizer. The rennet casein (low and medium concentrations) and glycerol films displayed higher OP's than those made with lactic acid casein and glycerol, and rennet casein (high protein content) and glycerol (Table 7). Protein to plasticizer ratio did not play a significant role in OP of the films.

Since these tests were evaluated at 0% R.H. we didn't see a possible effect of water to act as a further plasticizer. At elevated R.H.'s we could probably expect to see glycerol effect OP more than sorbitol because of it's greater affinity towards water. Due to these dry conditions OP differences based on protein concentration and type were not seen because proteins are generally not reactive with oxygen. OP for the films made with rennet and glycerol might have been elevated due to some trapped water, because these films did gel quicker than the others, especially the higher protein concentration films.

Table 6. Effect of Plasticizer Type on Oxygen Permeability (OP) of Casein-Based Edible Films (23°C, 0% R.H.)

Treatment[1] (Protein Powder%/ Plasticizer%)	Lactic Acid Casein[2,3]	Rennet Casein[2,3]
S(37.5/62.5)	0.653 ± 0.122^{a}	0.713 ± 0.155^{a}
S(50/50)	0.733 ± 0.133^{a}	1.017 ± 0.267^{a}
S(58.3/41.7)	0.813 ± 0.202^{a}	0.963 ± 0.153^{a}
G(37.5/62.5)	0.880 ± 1.101^{a}	7.057 ± 1.831^{b}
G(50/50)	2.177 ± 0.544^{b}	5.553 ± 2.842^{b}
G(58.3/41.7)	0.727 ± 0.272^{a}	1.837 ± 0.791^{a}

[1] Letter denotes plasticizer type: S=Sorbitol, G=Glycerol; Protein powder%/plasticizer% is reported as a dry basis.

[2] Different letters columnwise denote significant difference ($p<0.05$).

[3] Mean ± s.d. are reported as $cc \cdot \mu m/day \cdot m^2 \cdot kPa$.

Table 7. Effect of Protein Type on Oxygen Permeability (OP) of Casein-Based Edible Films (23°C, 0% R.H.)

Treatment[1] (Protein Powder%/ Plasticizer%)	Sorbitol[2,3]	Glycerol[2,3]
L(37.5/62.5)	0.653 ± 0.122^{a}	0.880 ± 1.101^{a}
L(50/50)	0.733 ± 0.133^{a}	2.177 ± 0.544^{a}
L(58.3/41.7)	0.813 ± 0.202^{a}	0.727 ± 0.272^{a}
R(37.5/62.5)	0.713 ± 0.155^{a}	7.057 ± 1.831^{b}
R(50/50)	1.017 ± 0.267^{a}	5.553 ± 2.842^{b}
R(58.3/41.7)	0.963 ± 0.153^{a}	1.837 ± 0.791^{a}

[1] Letter denotes casein type: L=Lactic acid casein, R=Rennet casein; Protein powder%/plasticizer% is reported as a dry basis.

[2] Different letters columnwise denote significant difference ($p<0.05$).

[3] Mean ± s.d. are reported as $cc \cdot \mu m/day \cdot m^2 \cdot kPa$.

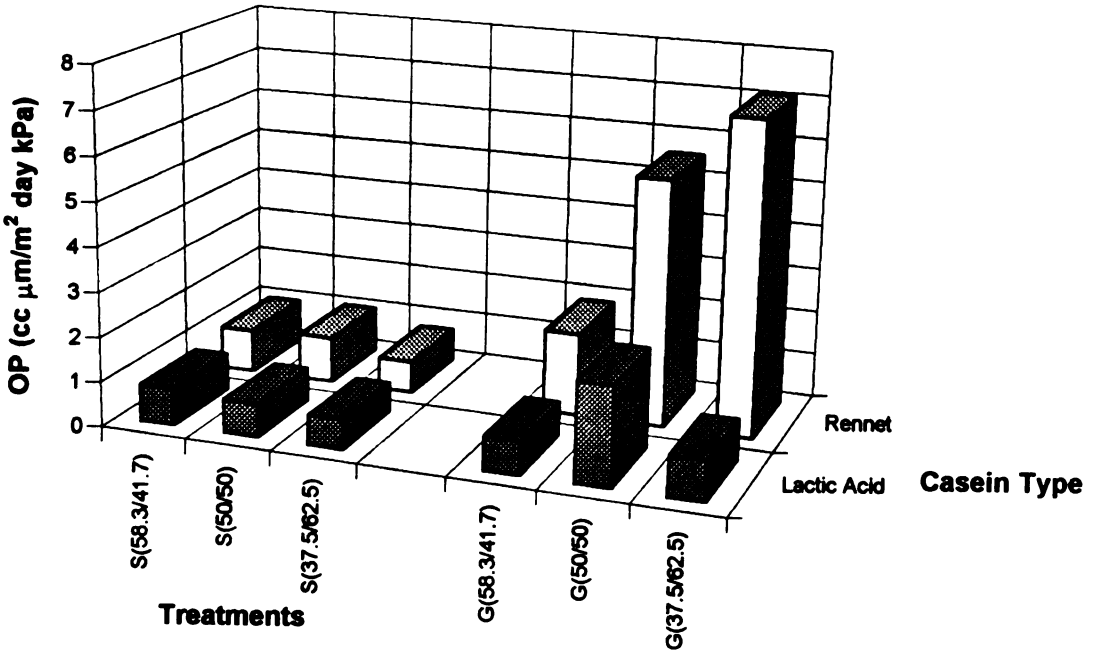


Figure 7. Oxygen Permeability (OP) of Casein-Based Edible Films ($23^{o}C$, 0% R.H.)

Treatments: S=Sorbitol, G=Glycerol;
(Protein Powder%/Plasticizer%)

Mechanical Properties

Tensile Strength (TS)

Upon conditioning, at 23°C and 50% R.H., films made with rennet casein and sorbitol had a white film layer form on their surface. It is not known what the white film that formed on the surface was, possibly a by-product of the hygroscopic properties of sorbitol. However, this film layer, did not seem to affect the TS of these films. Films made with rennet casein and sorbitol, 58.3% protein and 41.7% plasticizer, displayed a TS of 15.117 MPa, which was the highest observed. Films made with lactic acid casein and sorbitol, 37.5% protein and 62.5% plasticizer, displayed a TS of 0.415 MPa, which was the lowest observed (Table 8, 9; Figure 8). Films made with sorbitol as the plasticizer in all cases had significantly higher ($p<0.05$) TS's than those made with glycerol, at equal protein to plasticizer concentrations (Table 8). Rennet casein films tended to produce stronger films than lactic acid casein with either type of plasticizer, being more significant ($p<0.05$) at higher protein concentrations (Table 9). As Protein to plasticizer ratios increased TS increased significantly ($p<0.05$).

As expected films containing sorbitol displayed a higher TS than films formulated with glycerol, at the same protein to plasticizer contents. This is probably due to their greater crystallinity, and higher viscosity which it possesses, and the ability of glycerol to hold more water at equivalent R.H.'s, decreasing protein-protein interactions (Sicard and Leroy,1983). More calcium crosslinking, possibly being stronger than the direct protein-protein bonding, in the rennet casein films, because of the larger ash content, could be the main factor in the higher TS of rennet films compared to lactic acid films. The increase in

Table 8. Effect of Plasticizer Type on Tensile Strength (TS) of Casein-Based Edible Films (23°C, 50% R.H.)

Treatment[1] (Protein Powder%/ Plasticizer%)	Lactic Acid Casein[2,3]	Rennet Casein[2,3]
S(37.5/62.5)	2.427 ± 0.0751^{a}	3.827 ± 0.307^{a}
S(50/50)	7.483 ± 0.7457^{b}	9.527 ± 1.405^{b}
S(58.3/41.7)	11.647 ± 0.3800^{c}	15.117 ± 2.270^{c}
G(37.5/62.5)	0.415 ± 0.0603^{d}	0.830 ± 0.290^{d}
G(50/50)	1.243 ± 0.0273^{e}	2.423 ± 0.166^{ad}
G(58.3/41.7)	2.507 ± 0.0666^{a}	4.497 ± 0.698^{a}

[1] Letter denotes plasticizer type: S=Sorbitol, G=Glycerol; Protein powder%/plasticizer% is reported as a dry basis.

[2] Different letters columnwise denote significant difference ($p<0.05$).

[3] Mean ± s.d. are reported as MPa.

Table 9. Effect of Protein Type on Tensile Strength (TS) of Casein-Based Edible Films (23°C, 50% R.H.)

Treatment[1] (Protein Powder%/ Plasticizer%)	Sorbitol[2,3]	Glycerol[2,3]
L(37.5/62.5)	2.427 ± 0.075^{a}	0.415 ± 0.060^{a}
L(50/50)	7.483 ± 0.746^{b}	1.243 ± 0.047^{b}
L(58.3/41.7)	11.647 ± 0.380^{c}	2.507 ± 0.067^{c}
R(37.5/62.5)	3.827 ± 0.307^{a}	0.830 ± 0.290^{ab}
R(50/50)	9.527 ± 1.405^{b}	2.423 ± 0.166^{c}
R(58.3/41.7)	15.117 ± 2.270^{d}	4.497 ± 0.698^{d}

[1] Letter denotes casein type: L=Lactic acid casein, R=Rennet casein; Protein powder%/plasticizer% is reported as a dry basis.

[2] Different letters columnwise denote significant difference ($p<0.05$).

[3] Mean ± s.d. are reported as MPa.

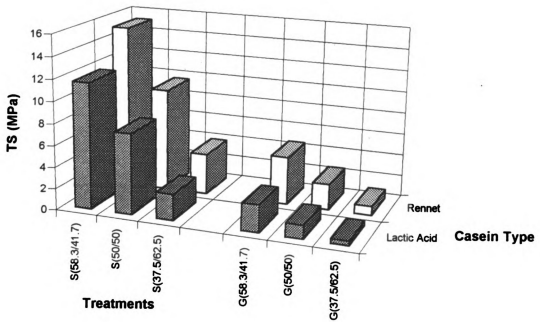


Figure 8. Tensile Strength (TS) of Casein-Based Edible Films ($23^{o}C$, 50% R.H.)
Treatments: S=Sorbitol, G=Glycerol;
(Protein Powder%/Plasticizer%)

TS as protein content increased is most likely due to an increase in protein-protein interactions.

Elongation (E%)

Again films made from rennet casein and sorbitol had a white film form on their surface upon conditioning, this appeared to make the film brittle reducing the E%. Films made with lactic acid casein and sorbitol, 50% protein and 50% plasticizer, displayed an E of 253.6%, which was the highest observed. Those made with rennet casein and sorbitol, 58.3% protein and 41.7% plasticizer, displayed an E of 17.9%, being the lowest observed (Table 10, 11; Figure 9). Films made with glycerol displayed significantly higher ($p<0.05$) E%'s than those made with sorbitol at the same protein to plasticizer ratio, except for the lactic acid casein (low protein content) and glycerol films which had a lower E% than lactic acid casein (low protein content) and sorbitol films (Table 10). Protein type did not contribute significantly to the E%, except when sorbitol was used. This being due to the white film that formed on the surface of the rennet casein and sorbitol films (Table 11). No significant trends in E% were present based on protein to plasticizer ratio.

Films containing glycerol had the higher E%, as expected, again because of the ability of glycerol to absorb more water than sorbitol, acting to further plasticize the films, making them less brittle. Due to the higher crystallinity present in sorbitol would tend to make them more rigid. However, as protein content increased a decrease in E% was not observed, which was not expected. It was thought that E% would decrease because of the increased protein-protein interactions increase, making them film more resistant to stretching.

Table 10. Effect of Plasticizer Type on Elongation (E%) of Casein-Based Edible Films (23°C, 50% R.H.)

Treatment[1] (Protein Powder%/ Plasticizer%)	Lactic Acid Casein[2,3]	Rennet Casein[2,3]
S(37.5/62.5)	170.7 ± 2.0^{a}	4.9 ± 9.8^{a}
S(50/50)	156.0 ± 6.1^{a}	7.6 ± 22.5^{b}
S(58.3/41.7)	50.6 ± 7.5^{a}	17.9 ± 4.6^{a}
G(37.5/62.5)	121.4 ± 10.2^{b}	123.2 ± 22.4^{c}
G(50/50)	253.6 ± 16.3^{c}	185.4 ± 22.8^{d}
G(58.3/41.7)	194.1 ± 20.6^{d}	223.5 ± 22.7^{e}

[1] Letter denotes plasticizer type: S=Sorbitol, G=Glycerol; Protein powder%/plasticizer% is reported as a dry basis.

[2] Different letters columnwise denote significant difference ($p<0.05$).

[3] Mean ± s.d. are reported as %.

Table 11. Effect of Protein Type on Elongation (E%) of Casein-Based Edible Films (23°C, 50% R.H.)

Treatment[1] (Protein Powder%/ Plasticizer%)	Sorbitol[2,3]	Glycerol[2,3]
L(37.5/62.5)	170.7 ± 2.0^{a}	121.4 ± 10.2^{a}
L(50/50)	156.0 ± 6.1^{a}	253.6 ± 16.3^{b}
L(58.3/41.7)	150.6 ± 7.5^{a}	194.1 ± 20.6^{cd}
R(37.5/62.5)	34.9 ± 9.8^{b}	123.2 ± 22.3^{a}
R(50/50)	77.6 ± 22.5^{c}	185.4 ± 22.8^{de}
R(58.3/41.7)	17.9 ± 4.6^{b}	223.5 ± 22.7^{bce}

[1] Letter denotes casein type: L=Lactic acid casein, R=Rennet casein; Protein powder%/plasticizer% is reported as a dry basis.

[2] Different letters columnwise denote significant difference ($p<0.05$).

[3] Mean ± s.d. are reported as %.

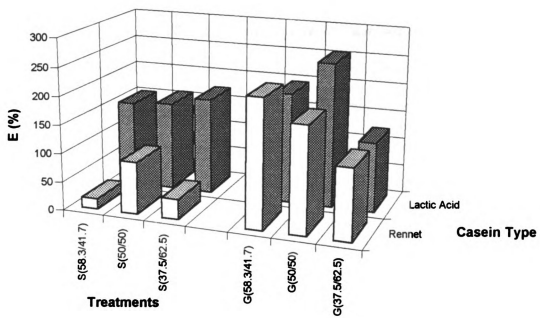


Figure 9. Elongation (E%) of Casein-Based Edible Films
($23^{O}C$, 50% R.H.)
Treatment: S=Sorbitol, G=Glycerol;
(Protein Powder%/Plasticizer%)

Comparisons to Other Films

Properties of casein-based edible films developed in this study compared favorably to synthetic polymers in some aspects, while in others they were inferior (Table 12). The casein-based films from this study included in Table 12 were chosen because they possessed good properties in the various categories. These casein-based films also compared favorably to other protein-based films that have been developed (Table 13). Casein-based films were very poor water vapor barriers as compared to synthetic films. Low density polyethylene (LDPE) is considered a good water barrier, while nylon 6 is considered rather poor one. The WVP of casein-based edible films is approximately 7 times greater than that of the nylon 6. This is most likely due to the hydrophilic characteristics that proteins possess. The water barrier properties of these films compared rather favorably to other edible protein films that have been developed. However, direct comparisons cannot be made due to the different experimental conditions, film composition, and thickness. The only protein-based films reported that possessed lower WVP's than our films were the caseinate films developed by Banerjee and Chen (1995) and Park and Chinnan (1990). However, films from those studies contained higher protein concentrations and tested under less severe conditions. Typically, as temperature or relative humidity rise so will the WVP.

The casein-based films developed in this study possessed good oxygen barrier properties compared to both synthetic and other protein-based edible films. Ethylene vinyl alcohol (EVOH) is considered a very good oxygen barrier and nylon 6 a good barrier. The OP of our casein-based films were comparable to that of EVOH. The OP's of our films were also lower than any of the values reported for other protein-based films. The

Table 12. Comparison of Selected Casein-Based Edible Films and Synthetic Polymers

Protein-Plasticizer[1]	Thickness (mm)	WVP[2] (g·mm/ m^2·d·kPa)	OP[3] (cc·μm/ m^2·d·kPa)	TS[4] (MPa)	E[4] (%)	Reference
LA-S Film (50/50)	0.203	45.03	0.71	7.48	156.0	Present
LA-G Film (58.3/41.7)	0.203	54.69	0.77	2.51	194.1	Present
R-S Film (37.5/62.5)	0.203	49.68	0.71	3.83	34.9	Present
R-G Film (50/50)	0.203	58.15	3.95	2.42	185.4	Present
Synthetic Polymers						
LDPE	0.0254	-	1870[4]	8.6-17	500	Salame (1986)
HDPE	0.0254	0.02	427[4]	17-35	300	Smith (1986)
EVOH (56% VOH)	0.0254	-	0.066	39.2-68.7	235-325	Foster (1986)
Nylon 6	0.0254	7.1	10.1	69-82.8	400-500	Tubrity & Sibilia (1986)

[1] Numbers in parenthesis denotes protein powder%/plasticizer %; L.A.=Lactic Acid Casein, R=Rennet Casein, S=Sorbitol, G=Glycerol, LDPE=Low density polyethylene, HDPE=High density polyethylene, EVOH=Ethylene vinyl alcohol (VOH-Vinyl alcohol).

[2] Evaluated at 37.8°C and 90% R.H.

[3] Evaluated at 23°C and 0% R.H.

[4] Evaluated at 23°C and 50% R.H.

Table 13. Comparisons of Various Protein-Based Edible Films

Film Type[1] (Protein-Plasticizer)	Thickness (mm)	WVP (g·mm/ m^2·d·kPa)	OP (cc·μm/ m^2·d·kPa)	TS[2] (MPa)	E[2] (%)	Reference
CZ-G (83.1/16.9)		7.69-11.49 (21°C, 85% R.H.)	13.0-44.9 (30°C, 0% R.H.)	-	-	Park and Chinnan (1990)
SPI-G (63.0/37.0)	0.064	-	4.75 (25°C, 0% R.H.)	3.13-5.23	66.5-90.3	Brandenburg *et al.* (1993)
WG-G (71.4/28.6)	0.140	108.4 (37.8°C, 100% R.H.)	3.82 (23°C, 0% R.H.)	1.8	25	Gennadios *et al.*(1993b) Aydt *et al. (1991)*
NFDM-G lactose extracted (75.0/25.0)	0.069	81.0 (30°C, 61% R.H.)	-	5.1	12.2	Maynes and Krochta (1994)
NFDM-G ultra-filtered (75.0/25.0)	0.071	70.3 (30°C, 65% R.H.)	-	9.1	5.2	Maynes and Krochta (1994)
WPI-G (62.5/37.5)	0.121	119.8 (25°C, 65% R.H.)	-	-	-	McHugh and Krochta (1994)
WPI-S (62.5/37.5)	0.129	61.92 (25°C, 79% R.H.)	-	-	-	McHugh and Krochta (1994)
WPI-G (70.0/30.0)	0.110	-	61.92 (23°C, 50% R.H.)	13.9	30.8	McHugh and Krochta (1994)
WPI-S (50.0/50.0)	0.110	-	8.3 (25°C, 79% R.H.)	14.7	8.7	McHugh and Krochta (1994)
SC-G (66.7/33.3)	0.109	12.90 (23°C, 72% R.H.)	-	2.98	29.89	Banerjee and Chen (1995)
CC-G (66.7/33.3)	0.105	7.91 (23°C, 72% R.H.)	-	4.25	1.45	Banerjee and Chen (1995)
α_{s1}-casein -G (98.0/2.0)	-	-	-	4.1	38.0	Motoki *et al.* (1987)
α_{s1}-casein -G transglut. (98.0/2.0)	-	-	-	10.6	77.0	Motoki *et al.* (1987)

[1] Numbers in parenthesis denotes protein %/plasticizer %; CZ=Corn Zein, SPI=Soy protein isolate, WG=Wheat gluten, NFDM=Non fat dry milk, WPI=Whey protein isolate, SC=Sodium caseinate, CC=Calcium caseinate, G=Glycerol, S=Sorbitol, transglut.=transglutaminase.

[2] Evaluated at 23°C and 50% R.H.

TS and E% of these casein-based films were considerably lower than the synthetic polymers. TS's were approximately 2 to 10 times weaker than the synthetic polymers, while the E% was approximately 1.5 to 2 times lower. These casein-based films were very comparable to the other protein-based films in TS, while they possessed much higher E%'s than any of the reported protein-based edible films.

Storage Study

Moisture Content

The casein-based edible films proved to be ineffective in preventing moisture loss in processed cheese slices. Moisture content of the cheese began at 39.32%, and those slices wrapped in LDPE did not lose any significant amount of moisture over the duration of storage. However, cheese slices wrapped in the casein-based films and the unwrapped controls lost a significant amount ($p<0.05$) of moisture, dropping to about 30% (Table 14). In fact, these films didn't even delay the loss of moisture. Both casein-based wraps performed similarly to unwrapped controls, in retaining moisture in the cheese slices, throughout storage (Table 15). This can be attributed to the poor moisture barrier properties these films possess. Also, this was probably a worst case scenario for evaluating these films due to the high moisture content of the cheese, and because of the high R.H. of the storage environment, which was 88%.

There was a dramatic increase in the moisture content of the casein-based films, when used as a cheese wrap (Table 16). The lactic acid casein and sorbitol film had a moisture content of 9.02%, while the lactic acid casein and glycerol film had an initial moisture

Table 14. Effect of Storage Time on Moisture Content of Processed Cheese Slices Packaged in Various Wraps (2.2°C, 88% R.H.)

Percent Moisture				
	Wrap Type			
Storage (Days)	**LDPE[1,2,3]**	**L.A.-S Film (50/50) [1,2,3]**	**L.A.-G Film (58.3/41.7) [1,2,3]**	**No Wrap [1,2,3]**
0	39.32 ± 0.64^{a}	39.32 ± 0.64^{a}	39.32 ± 0.64^{a}	39.32 ± 0.64^{a}
5	39.62 ± 4.77^{a}	31.10 ± 2.23^{b}	34.04 ± 0.54^{b}	30.75 ± 4.77^{b}
10	41.41 ± 1.39^{a}	28.19 ± 0.44^{bc}	32.32 ± 0.48^{bc}	29.39 ± 0.88^{b}
15	41.86 ± 0.39^{a}	28.01 ± 2.39^{bc}	27.73 ± 1.20^{c}	29.69 ± 0.22^{b}
20	40.29 ± 1.00^{a}	26.48 ± 0.75^{c}	31.55 ± 0.69^{bc}	28.24 ± 1.71^{b}
25	39.59 ± 0.23^{a}	25.70 ± 0.64^{c}	29.30 ± 0.91^{c}	28.86 ± 0.77^{b}
30	42.75 ± 0.15^{a}	28.92 ± 2.18^{bc}	29.85 ± 1.35^{c}	30.77 ± 0.51^{b}

[1] LDPE=Low density polyethylene, L.A.=Lactic acid casein, S=Sorbitol, G=Glycerol; numbers in parenthesis (protein powder%/plasticizer%).

[2] Different letters columnwise denotes a significant difference ($p<0.05$).

[3] Mean ± s.d.

Table 15. Effect of Wrap Type on Moisture Content of Processed Cheese Slices During Storage (2.2°C , 88% R.H.)

Percent Moisture							
	Storage Time (Days)						
Wrap Type[1]	0[2,3]	5[2,3]	10[2,3]	15[2,3]	20[2,3]	25[2,3]	30[2,3]
LDPE	39.32 ± 0.64[a]	39.62 ± 4.77[a]	41.41 ± 1.39[a]	41.86 ± 0.39[a]	40.29 ± 1.00[a]	39.59 ± 0.23[a]	42.75 ± 0.15[a]
L.A.-S Film (50/50)	39.32 ± 0.64[a]	31.10 ± 2.23[b]	28.19 ± 0.44[b]	28.01 ± 2.39[b]	26.48 ± 0.75[b]	25.70 ± 0.64[b]	28.92 ± 2.18[b]
L.A.-G Film (58.3/41.7)	39.32 ± 0.64[a]	34.04 ± 0.54[b]	32.32 ± 0.48[b]	27.73 ± 1.20[b]	31.37 ± 0.69[c]	29.30 ± 0.91[b]	28.85 ± 1.35[b]
No Wrap	39.32 ± 0.64[a]	30.75 ± 1.03[b]	29.39 ± 0.88[b]	29.69 ± 0.22[b]	28.24 ± 1.71[c]	28.86 ± 0.77[b]	30.77 ± 0.51[b]

[1] LDPE=Low density polyethylene, L.A.=Lactic acid casein, S=Sorbitol, G=Glycerol; numbers in parenthesis (protein powder%/plasticizer%).

[2] Different letters columnwise denotes a significant difference ($p<0.05$).

[3] Mean ± s.d.

Table 16. Effect of Storage Time on Moisture Content of Edible Films Used as a Wrap for Processed Cheese Slices (2.2°C, 88% R.H.)

	Percent Moisture	
	Wrap Type	
Storage (Days)	L.A.-S Film (50/50) [1,2,3]	L.A.-G Film (58.3/41.7) [1,2,3]
0	9.02 ± 1.21[a]	17.80 ± 3.86[a]
5	37.60 ± 2.58[b]	45.47 ± 1.59[b]
10	37.85 ± 3.76[b]	42.73 ± 1.43[bc]
15	36.69 ± 2.73[b]	37.46 ± 0.72[c]
20	37.96 ± 1.08[b]	44.12 ± 1.71[bc]
25	37.29 ± 0.12[b]	43.10 ± 0.24[bc]
30	35.72 ± 4.16[b]	38.14 ± 4.27[c]

[1] L.A.=Lactic acid casein, S=Sorbitol, G=Glycerol; numbers in parenthesis (protein powder%/ plasticizer%).

[2] Different letters columnwise denotes a significant difference ($p<0.05$).

[3] Means ± s.d.

content of 17.80%. In the first 5 days of storage these quickly rose to 37.60% and 45.47% for the casein and sorbitol, and casein and glycerol films respectively, then stayed relatively constant throughout the rest of storage. Which is due to the hydrophilic nature of both the casein and the plasticizer (Swaisgood, 1985; Sicard, 1982). This was the same trend that was observed with the moisture loss of the cheese slices. If we look at the weight change of the cheese slices and wrap (package system) over the period of storage we see that the water lost by the cheese in retained in the film (Table 17). The package system of cheese slices with the casein and sorbitol film maintained a constant weight throughout storage, while the package system of the cheese slices wrapped with the casein and glycerol film actually gained weight during storage. This is most likely attributed to the ability of glycerol to absorb moisture from the air (Merck, 1989).

Color

Casein-based films were ineffective at retaining color in processed cheese slices also (Table 18). Cheese slices wrapped in LDPE retained their original, creamy orange, appearance throughout the duration of storage. A significant change ($p<0.05$) was observed in the Hunter L-Value for cheese slices wrapped in the casein-based films and the unwrapped controls occurring shortly after being packaged (Table 18a). This value shows a darkening of the cheese, decreasing in value from 71.07 to about 56.0. There was no significant difference in L-Value among the cheese slices wrapped in the casein-based films and the unwrapped slices. The unwrapped and casein-based film wrapped slices also witnessed a significant change ($p<0.05$) in redness during storage (Table 18b). This is shown by the a-value increasing from 8.60 to around 11.0, indicating a slight

Table 17. Weight Gain of Processed Cheese Slices and Wrap During Storage (2.2°C, 88% R.H.)

Storage (Days)	LDPE[1,2,3]	L.A.-S Film (50/50) [1,2,3]	L.A.-G Film (58.3/41.7) [1,2,3]
0	0.000 ± 0.000[a]	0.000 ± 0.00[a]	0.000 ± 0.00[a]
5	0.016 ± 0.002[a]	0.731 ± 0.17[a]	3.342 ± 0.76[b]
10	0.017 ± 0.003[a]	-0.054 ± 0.37[a]	2.791 ± 0.77[b]
15	0.005 ± 0.002[a]	0.383 ± 1.31[a]	3.587 ± 1.59[b]
20	-0.026 ± 0.035[a]	0.995 ± 0.03[a]	4.148 ± 0.45[b]
25	0.020 ± 0.017[a]	0.206 ± 0.39[a]	2.856 ± 1.02[b]
30	-0.006 ± 0.004[a]	0.600 ± 0.94[a]	1.916 ± 1.06[b]

[1] LDPE=Low density polyethylene, L.A.=Lactic acid casein, S=Sorbitol, G=Glycerol; numbers in parenthesis (protein powder%/plasticizer%).

[2] Different letters columnwise denotes a significant difference ($p<0.05$).

[3] Means ± s.d. reported in grams.

Table 18. Effect of Wrap Type on Color Changes in Processed Cheese Slices During Storage (2.2°C, 88% R.H.)

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

Wrap Type[1]	Storage Time (Days)						
	0[2,3]	5[2,3]	10[2,3]	15[2,3]	20[2,3]	25[2,3]	30[2,3]
LDPE	71.07 ± 0.60[a]	70.80 ± 0.95[a]	71.33 ± 1.36[a]	71.37 ± 0.50[a]	69.97 ± 0.15[a]	69.80 ± 0.35[a]	69.70 ± 0.60[a]
L.A.-S Film (50/50)	71.07 ± 0.60[a]	59.73 ± 1.50[b]	57.87 ± 0.47[b]	58.07 ± 3.89[b]	56.20 ± 1.32[b]	54.93 ± 1.10[b]	55.53 ± 3.36[b]
L.A.-G Film (58.3/41.7)	71.07 ± 0.60[a]	67.17 ± 1.19[c]	63.70 ± 1.22[c]	57.33 ± 2.82[b]	63.33 ± 2.11[c]	60.90 ± 1.56[c]	58.13 ± 2.42[b]
No Wrap	71.07 ± 0.60[a]	61.30 ± 0.60[b]	60.53 ± 1.21[bc]	60.33 ± 0.32[b]	56.10 ± 2.86[b]	55.67 ± 0.80[b]	55.23 ± 1.45[b]

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

Wrap Type[1]	Storage Time (Days)						
	0[2,3]	5[2,3]	10[2,3]	15[2,3]	20[2,3]	25[2,3]	30[2,3]
LDPE	8.60 ± 0.35[a]	9.10 ± 0.36[a]	8.93 ± 0.38[a]	8.33 ± 0.61[a]	9.00 ± 0.70[a]	8.70 ± 0.10[a]	8.10 ± 0.72[a]
L.A.-S Film (50/50)	8.80 ± 0.35[a]	10.67 ± 1.04[b]	11.57 ± 0.15[b]	10.57 ± 0.55[b]	10.03 ± 0.21[a]	11.47 ± 0.75[b]	10.93 ± 0.61[b]
L.A.-G Film (58.3/41.7)	8.60 ± 0.35[a]	10.43 ± 0.31[b]	10.33 ± 0.47[b]	11.43 ± 0.65[b]	9.60 ± 0.27[a]	11.13 ± 0.47[b]	10.63 ± 0.21[b]
No Wrap	8.60 ± 0.35[a]	11.30 ± 0.44[b]	11.30 ± 0.76[b]	10.73 ± 0.31[b]	11.67 ± 0.81[b]	12.10 ± 0.46[b]	11.43 ± 0.61[b]

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

Wrap Type[1]	Storage Time (Days)						
	0[2,3]	5[2,3]	10[2,3]	15[2,3]	20[2,3]	25[2,3]	30[2,3]
LDPE	33.93 ± 0.58[a]	34.60 ± 0.17[a]	34.87 ± 0.55[a]	33.90 ± 0.27[a]	34.30 ± 0.10[a]	34.37 ± 0.06[a]	34.77 ± 0.49[a]
L.A.-S Film (50/50)	33.93 ± 0.58[a]	33.50 ± 0.87[a]	32.47 ± 0.65[a]	32.00 ± 1.83[a]	31.30 ± 0.61[b]	30.87 ± 0.74[b]	31.10 ± 1.74[b]
L.A.-G Film (58.3/41.7)	33.93 ± 0.58[a]	35.30 ± 0.53[a]	33.73 ± 1.42[a]	31.77 ± 1.30[a]	33.40 ± 1.32[ab]	33.27 ± 0.71[ab]	32.20 ± 1.14[ab]
No Wrap	33.93 ± 0.58[a]	33.73 ± 1.59[a]	34.10 ± 1.33[a]	33.77 ± 0.59[a]	31.77 ± 1.16[b]	32.13 ± 0.71[ab]	31.77 ± 0.67[b]

[1] LDPE=Low density polyethylene, L.A.=Lactic acid casein, S=Sorbitol, G=Glycerol; numbers in parenthesis (protein powder%/plasticizer%).

[2] Different letters columnwise denotes a significant difference ($p<0.05$).

[3] Means ± s.d.

reddening of the cheese. No significant difference was observed between the cheese slices wrapped in the casein-based film or the unwrapped slices, again occurring shortly after being packaged. A significant change ($p<0.05$) in Hunter b-value occurred towards the end of storage in cheese slices wrapped in the casein-based films and the unwrapped controls (Table 18c). Values decreased from 33.93 to around 32.0 at the 20 day period and remaining relatively constant, indicating a slight loss of yellowness in the cheese. This color change is attributed to the loss of moisture in the cheese slices, because this color change (L-value and a-value) occurred at the same rate as did moisture loss. As with moisture loss following the initial change values tended to remain constant for the rest of storage.

The color of the casein-based film itself after being used to wrap processed cheese slices changed significantly ($p<0.05$) during storage (Table 19). Casein-based films were significantly more transparent ($p<0.05$) than the LDPE film. This is shown by the Hunter L-value at day 0 of storage, where LDPE has a value of 23.63 and casein-based films have values of 13.40 and 11.83 for the films containing sorbitol and glycerol, respectively (Table 19a), values closer to 0 being more transparent since the black tile was used as the background tile. However, by the end of storage there was no difference in transparency among the films. Hunter a-value were similar for all the films and remained constant throughout storage (Table 19b). Films at the beginning of storage were similar (being slightly bluish), with the casein-based films significantly changing ($p<0.05$) to a slight yellowish color during storage, while the LDPE films remained unchanged (Table 19c). This is observed in the similar changes of the Hunter b-values of the casein-based films

Table 19. Comparison of Color Changes of Wraps After Storage on Processed Cheese Slices (2.2°C, 88% R.H.)

a) L-Value (0 transparent to 100 white)

	Storage Time (Days)						
Wrap Type[1]	**0[2,3]**	**5[2,3]**	**10[2,3]**	**15[2,3]**	**20[2,3]**	**25[2,3]**	**30[2,3]**
LDPE	23.63 ± 0.35[a]	-	-	-	-	-	24.47 ± 0.75[a]
L.A.-S Film (50/50)	13.40 ± 1.64[b]	20.60 ± 1.05[a]	21.93 ± 2.12[a]	21.93 ± 1.35[a]	20.33 ± 1.52[a]	20.93 ± 0.67[a]	20.83 ± 2.97[a]
L.A.-G Film (58.3/41.7)	11.83 ± 1.07[b]	21.17 ± 1.37[a]	22.90 ± 1.77[a]	19.17 ± 3.19[a]	20.70 ± 1.18[a]	21.67 ± 2.71[a]	21.67 ± 3.36[a]

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

	Storage Time (Days)						
Wrap Type[1]	**0[2,3]**	**5[2,3]**	**10[2,3]**	**15[2,3]**	**20[2,3]**	**25[2,3]**	**30[2,3]**
LDPE	0.13 ± 1.25[a]	-	-	-	-	-	0.67 ± 1.36[a]
L.A.-S Film (50/50)	-0.80 ± 0.36[a]	-0.30 ± 0.20[a]	-0.60 ± 0.85[a]	-0.567± 0.32[a]	0.67 ± 0.29[a]	0.33 ± 0.21[a]	-0.37 ± 0.50[a]
L.A.-G Film (58.3/41.7)	-0.50 ± 0.30[a]	-0.367 ± 0.45[a]	-0.53 ± 0.45[a]	-0.87 ± 0.57[a]	-0.33 ± 0.25[a]	-0.73 ± 0.16[a]	-0.23 ± 0.58[a]

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

	Storage Time (Days)						
Wrap Type[1]	**0[2,3]**	**5[2,3]**	**10[2,3]**	**15[2,3]**	**20[2,3]**	**25[2,3]**	**30[2,3]**
LDPE	-1.47 ± 0.85[a]	-	-	-	-	-	-1.73 ± 0.90[a]
L.A.-S Film (50/50)	-1.87 ± 0.85[a]	0.50 ± 0.20[a]	0.30 ± 0.446[a]	0.60 ± 0.44[a]	-0.60 ± 0.61[a]	0.10 ± 0.36[a]	0.63 ± 0.51[b]
L.A.-G Film (58.3/41.7)	-1.50 ± 0.78[a]	0.50 ± 0.56[a]	0.73 ± 0.60[a]	0.57 ± 0.35[a]	0.30 ± 0.20[a]	0.73 ± 0.21[a]	0.67 ± 0.21[b]

[1] LDPE=Low density polyethylene, L.A.=Lactic acid casein, S=Sorbitol, G=Glycerol; numbers in parenthesis (protein powder%/plasticizer%).

[2] Different letters columnwise denotes a significant difference ($p<0.05$).

[3] Means ± s.d.

from about -1.50 to about 0.65. These color changes in the film might be attributed to some residual cheese sticking to the film.

CONCLUSIONS

1. Casein-based films developed in this study possess poor water barrier properties.
2. They do possess good oxygen barrier properties, similar to synthetic polymers with good oxygen barrier properties.
3. Sorbitol used as a plasticizer will provide films with better overall properties than if glycerol were used as the plasticizer.
4. Casein-based films tend to have inferior properties compared to synthetic films, except for oxygen barrier properties.
5. Overall properties of casein-based films from this study compare favorably to properties of other protein-based films.
6. These casein-based films did not act as good wrap for processed cheese slices.

RECOMMENDATIONS

Work needs to be done on these films to improve their WVP properties. This could be accomplished with the use of crosslinking agents, or the incorporation of lipids into the films or as part of a bilayer film. Studies need to be done to determine the properties of these films at different environmental conditions, temperature and R.H.. Biodegradation studies should be conducted to determine biodegradability of these films and to establish testing methods for this. Further studies need to be done incorporating these films into other food systems, especially oxygen sensitive food items where these films could provide their greatest utility. Processes to seal these films must also be developed if they are to be used as an alternative packaging material.

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