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DEVELOPMENT AND CHARACTERIZATION
OF CASEIN-WAX-BASED EDIBLE FILMS

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DEVELOPMENT AND CHARACTERIZATION OF CASEIN-WAX EDIBLE FILMS

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ABSTRACT

DEVELOPMENT AND CHARACTERIZATION OF CASEIN-WAX EDIBLE FILMS

By

Jay Lyle Chick

This study was conducted to develop and characterize edible films produced from lactic acid casein and either carnauba or candelilla wax. Films were produced by mixing lactic acid casein and sorbitol in distilled water at a pH of 10, then adding either melted carnauba or candelilla wax, followed by homogenization. Film microstructure was viewed using scanning electron microscopy, and differential scanning calorimetry was used to evaluate thermal properties of the films and film forming components. Water and oxygen barrier properties, as well as the mechanical properties of these films were evaluated over various wax contents and relative humidities.

Films were found to be continuous and homogeneous with an increasing separation of wax within the film structure as it's content increased. Films with candelilla wax incorporated were found to provide the best water barrier. However, water barrier properties of these films were still poor compared to synthetic polymers. These films provided a good oxygen barrier, and were found not to be affected by the incorporation of either carnauba or candelilla wax. Incorporation of wax increased tensile strength while decreasing elongation compared to films without wax, these properties also being lower than synthetic polymers. A good, but weak, heat seal was able to be made around the melt temperatures of lactic acid casein and sorbitol.

Dedicated to the memory of my Grandparents,
Dr. O.B. and Bernice McGillicuddy and Herman and Leeta Chick,
for showing me that hard work, education, and kindness are keys to success,
and to my fiancee Heather for all of her love and support

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INTRODUCTION

In 1909 Dr. Leo Hendrick Baekland reacted phenol and formaldehyde together to form the first synthetic plastic called Bakelite. Soon to follow was the development of many other new synthetic polymers, like cellulose acetate and polyvinyl chloride in 1927. Polyethylene, today's major food packaging plastic, was developed in 1935 by Imperial Chemical Industries in England (Hanlon, 1992). Synthetic polymers now have been developed that provide good barriers against vapor migration, possess good mechanical properties, are transparent or opaque, and can be printed upon. As these properties have been developed to perform useful functions for packaging their use has become widespread. Although, natural plastics have been always used in some beneficial way, recently edible natural polymers have gained increased attention in the food and packaging industries, due to their value added properties they can impart.

Edible films have been used for centuries for longer food preservation, such as the coating of fruits with wax or coating foods with lard. Edible films have a number of advantages. They can enhance functional and nutritional properties of a food, they can be used to protect small pieces or portions of food, they can be used inside heterogeneous foods to separate components, since they are edible there is the opportunity for little to no waste being generated by allowing for the change of a multilayer plastic to a single, recyclable layer, for example (Krochta and DeMulder-Johnston, 1997; Guilbert, 1986).

Natural edible films properties differ from those of synthetic materials, especially mechanically, where they are weaker. However, edible films show other properties that make them useful materials. The advantages of natural edible films are associated with lowering the migration of moisture, fats, and oils from food. Edible films 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 naturally occurring high molecular weight molecules, these provide 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: hydrocolloids, lipids, and a composite of both a hydrocolloid and lipid. Composite films are either found as a bilayer (laminate) structure or as an emulsion. Hydrocolloids can be either proteins or polysaccharides, like corn, soy, wheat, and milk proteins, or pectin, starch, and cellulose derivatives (Kester and Fennema, 1986). Lipids used are fatty acids, fatty alcohols, or materials consisting of a combination of these, such as acetoglycerides, surfactants, and waxes. Edible films from protein are good oxygen barriers but poor water barriers, while lipid films have been shown to be good water barriers and poor oxygen barriers.

This research was to develop emulsified edible films using lactic acid casein, either carnauba wax or candelilla wax, and sorbitol as a plasticizer. The intention was to combine the positive qualities that the protein and lipid materials possess into one film. Lactic acid casein produces transparent films with very good oxygen barrier properties (Chick, 1996), while carnauba and candelilla wax are good water barriers and also have a GRAS status (Donhowe and Fennema, 1993). Lactic acid casein, carnauba wax, and candelilla wax were chosen for this study based on past studies showing that films produced from these components displayed better than films produced from rennet casein, beeswax, or acetylated monoglyceride (Chick, 1996; Donhowe and Fennema, 1993). The objectives of this research were:

1. To develop optimal formulations and processes in the formation of lactic acid casein, and carnauba or candelilla wax emulsion films.
2. To study some of the basic characteristics of these films, including their microstructure and thermal properties.
3. To study the water and oxygen barrier properties of these emulsion films.
4. To study the mechanical properties (tensile strength, elongation, and heat sealing qualities) of these emulsion films.

1.0 LITERATURE REVIEW

1.1 Formation of Edible Films and Coatings

There are three basic steps usually followed in the formation of a protein edible film or coating. Mixing of the film forming constituents in a solvent is the first step, to obtain a dispersion of the high molecular weight polymer. This is followed by casting or spreading a thin layer of the film forming solution onto a smooth level surface or the food item. A drying process then takes place to allow the solvent to evaporate, allowing the protein to form a matrix and subsequently the free standing film or coating (Cuq *et al.*, 1995).

1.1.1 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). Although the molecular weight of casein (≈20,000 daltons) is lower than other polymers, like wheat gluten (50,000-100,000 daltons)(Gennadios and Weller, 1990) or high density polyethylene (100,000 - 250,000 daltons)(Smith, 1986), it is still high enough to form a polymer matrix. Two types of high molecular weight polymers are used in the formation of edible films and coatings, 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 which 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.

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. Water and ethanol have been the two primary solvents used (Kester and Fennema, 1986).

A number of additives can also be incorporated into the film or film forming solution that alter the properties of the edible film or coating. Their 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 emulsion 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, and sorbitol. 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 delay the degradation of the food product 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 have included sorbic acid and potassium sorbate among others.

1.1.2 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. 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. 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; Kester and Fennema, 1986).

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).

1.2 Composition and Properties of Components Used in Edible Films

The composition, structure, and characteristics of the components that make up the edible film will effect the ultimate properties of that film. Casein and waxes have been used as high molecular weight polymers in edible films, while sorbitol has been a common plasticizer.

1.2.1 Casein

Natural liquid milk contains 3.3-3.9% protein, of which 20% are whey proteins and 80% are casein proteins. The casein proteins are comprised of α_{s1}-, α_{s2}-, β-, κ-, and γ-casein (Table 1). Casein is generally characterized as being mainly random coil in structure, amphiphilic (containing both hydrophobic and hydrophilic regions), with the presence of phosphoseryl groups able to bind calcium. Two of the major casein types, lactic acid casein and rennet casein, are precipitated from milk by the use of lactic acid or rennet (chymosin). Lactic acid casein is characterized by an isoelectric point (pI) of 4.6, while rennet casein has a pI near 7.0 (Southward and Walker, 1980). Primary active groups in casein consist of the peptide, amido, and carboxyl groups present at a 832, 114, and 99 mole equivalence per 100 kg (Appendix A)(Mark *et al.*, 1989a). Casein has been commonly used as adhesives, sizing agents, and as stabilizers/emulsifiers in foods or paints. The properties of casein have been reviewed by Leman and Kinsella (1989), Swaisgood (1985), Kinsella (1984), Dalgleish (1982), and Fox and Mulvihill (1982).

Table 1. Casein Composition

Casein Fraction	**% Total Casein**	**Amino Acids**	**Phosphoseryl Groups**	**Molecular Wt. (Daltons)**
α_{s1}-	34-45	199	8-9	23,000
α_{s2}-	10-12	207	10-13	23,000-25,000
β-	31-36	209	5	23,900-23,980
γ-	3-5			
γ_1-		181	1	20,520
γ_2-		104	0	11,822
γ_3-		102	0	11,557
κ-	10-13	169	1	19,000

1.2.1.1 α_{s1}- and α_{s2}-casein

On average, 38-45% of casein is comprised of α_{s1}-casein. This protein has been determined to be 199 amino acid units long (Appendix B), with a calculated molecular weight of about 23,000 daltons, depending on the variant. Amino acids are monomers of the protein molecule, consisting of at least one primary amino group (-NH_2) and one carboxyl group (-COOH), with a side chain (R) of variable composition (Appendix A). There are 20 amino acid types commonly found in proteins.

The C-terminal of α_{s1}-casein, the end of the amino acid chain with the carboxyl group, is comprised of some α–helices, β-sheets, and β-turns, with the rest being random coil in structure. The α-helix is a stable structure with 3.6 amino acids per helical turn, the side chains point outward. β-sheets are zigzag structures, where chains of amino acids are linked together by interchain hydrogen bonds forming the sheet. β-turns occur when a polypeptide chain bends over itself forming the turn, with it's stability provided by a hydrogen bond. The N-terminal, the end of the amino acid chain with the amino group, is mainly random coil in structure. Secondary structure is limited due to the presence of proline residues, making up about 8.5% of the residues. Proline, because of it's electrostatic and steric properties, interrupts helices, giving rise to bending, and cannot be integrated into β structures (Appendix A).

There are two variants of this protein, the difference being in the number of phosphoseryl groups, either 8 or 9. These phosphoseryl groups allow the protein to bind Ca^{2+}. This calcium binding can aid in strengthening the protein matrix. α_{s1}-casein can bind up to 8-10 moles Ca^{2+}/mole protein under normal circumstances and up to 20 mole Ca^{2+}/mole protein at high Ca^{2+} concentrations. All but one of the phosphoseryl groups, in both variants, are found between amino acids 41 and 80, closer to the N-terminal, contributing to it's random coil nature. Three hydrophobic regions are present in α_{s1}-casein, from amino acid units 1 to 40, 90 to 110, and 130 to 199. The overall charge being -21mV at pH 6.8, with a hydrophobicity of 1172 cal/residue.

The α_{s2}-caseins comprise 10-12% of the casein proteins. It consists of a 207 amino acid unit chain (Appendix B), with a calculated molecular weight of 23,000-25,000 daltons. Again with the presence of proline, about the same amount as α_{s1}-casein, any secondary structure is limited.

There are 4 variants of α_{s2}-casein, again differing in the number of phosphoseryl groups, ranging from 10-13. These phosphoseryl groups are fairly evenly distributed throughout the protein. A hydrophobic region is found at the C-terminal, between amino acid units 160 and 207. 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.

1.2.1.2 β- and γ-casein

β-casein makes up 31-36% of the casein proteins. This protein is 209 amino acid in length (Appendix B), with a molecular weight of 23,900-23,980 daltons. 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. There are five phosphoseryl groups which are all located in the N-terminal. It can bind 4-5 mole Ca^{2+}/mole protein. β-casein is the most hydrophobic of the casein proteins, with an overall hydrophobicity of 1334 cal/residue. The hydrophobic C-terminal, between amino acid residues 30 to 209, has a hydrophobicity of 1408 cal/residue, and carries a net charge of -12mV at pH 6.8.

β-casein can undergo proteolysis at 1 of 3 lysine residues, 28, 104, and 106, producing a pair of polypeptide chains, of which one half is lost into the milk serum, with the other half making up γ-casein, representing 3-5% of the total casein fraction.. The variants of this protein have amino chain lengths of 181, 104, and 102 residues (Appendix B), with molecular weights of 20,520, 11,822, and 11,557 daltons respectively. Of these only the largest contains a phosphoseryl group.

1.2.1.3 κ-casein

The final casein protein is κ-casein, making up 10-13% of the total. It contains 169 amino acid units (Appendix B), with a molecular weight of 19,000 daltons. Loucheaux-Lefebvre *et al.* (1978) determined that κ-casein consisted of 26% α-helix, 31% β-sheet, and 24% β-turns. They determined that amino acid residues 105 and 106 form either an α-helix or β-sheet between two stable β-turns and another β-turn at residues 113 to 116, making that linkage accessible to proteolysis by rennet (or chymosin). There is only one phosphoseryl group and two cysteine residues. κ-casein can contain from 0-3 oligosaccharide chains. These oligosaccharides can attach at the threonine residues located at amino acid chain units 131, 133, and 135. The primary initial point of attachment being at threonine 133. Due to the presence of only 1 phosphoseryl group κ-casein is able to bind only 1-2 mole Ca^{2+}/mole protein. The overall hydrophobicity is 1224 cal/residue, with an overall charge of -4 mV, at pH 6.8.

1.2.2 Waxes

Waxes are described as being simple lipids comprised of the combination of a long chain alcohol and a long chain fatty acid (Nawar, 1984). Carnauba and candelilla wax are two waxes with a Generally Regarded As Safe (GRAS) status. They both are derived from plant sources. Properties of carnauba and candelilla wax have been reviewed by Mark *et al.* (1989b), Howe-Grant (1984), Bennett (1975), and Warth (1956).

1.2.2.1 Candelilla Wax

Candelilla wax is obtained from either of two small reed-like plants, the *Euphorbia celifera* Alcocer or the *E. antisyphilitica* Zuccarini, found in Mexico and Southwest Texas. It has been commonly used in products like chewing gum and cosmetics.

Candelilla wax is comprised of 28-29% wax esters, these being simple or hydroxylated. In simple wax esters the side chains are comprised of a ethyl groups. It contains 12-14% alcohols, sterols, and resins, 49-51% hydrocarbons (29,31, or 33 carbon units long). Straight chain or cyclic free acids constitute 7-9%, with a moisture content of 0.5 - 3% and 0.7-1% inorganic residue (Table 2).

Candelilla wax has a melting point ranging from 68.5-72.5°C and a flash point of 241°C. Candelilla wax is reported as being a fairly hard material. It's saponification number ranges from 43-65, this being mg's of potassium hydroxide (KOH) per gram of sample to neutralize any free acids and saponify the ester linkages. The acid number, the amount (mg's) of KOH per gram of sample to neutralize any free acids, ranges from 12-22, with the ester number ranging from 31-43, this being the difference between the saponification number and acid number. The iodine value is reported to range from 15-44, this being a measure of unsaturated linkages, reported as percent iodine absorbed.

1.2.2.2 Carnauba Wax

Carnauba wax is obtained from a palm tree, *Copernica cerifera* Martius, found in Brazil and the northern parts of Argentina and Paraguay. It has been commonly used for polishes, candles, candies, and cosmetics. Carnauba wax is comprised of 84-85% alkyl esters of wax acids, these being simple, acid, hydroxylated acid esters or diesters. It contains 2-3% polyhydric or oxy-alcohols, 4-6% resins, and 2-3% lactides. 1.5-3% is comprised of hydrocarbons (27, 29, and 31 carbons in length), 3-3.5% free wax acids (carnaubic or cerotic), and 0.5-1% moisture and mineral matter (Table 2).

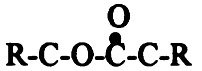
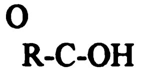
It has a melting point of 82.5-86°C, and a flash point ranging from 298-310°C. Carnauba wax is also considered a hard material. The saponification number ranged from 70-88, with an acid number ranging from 2-10, and and ester number between 75 and 88. Carnauba wax has an iodine value between 7 and 14.

1.2.3 Plasticizers

Sorbitol is a naturally occurring carbon backboned polyhydric alcohol. Besides use as a plasticizer it is often used as a sweetener, humectant, and pharmaceutic aid. Properties of sorbitol have been reviewed by Merck (1989), Sicard and Leroy (1983), and Sicard (1982).

Sorbitol was discovered in 1872 in the berries of mountain ash (*Sorbus aucuparia*

Table 2. Composition of Candelilla and Carnauba Wax

		Composition of Waxes	
	Structure	**Candelilla Wax**	**Carnauba Wax**
Wax esters	 $R\text{-}C\text{-}O\text{-}\overset{O}{C}\text{-}C\text{-}R$	28-29	84-85
simple		20-21	5-6
acid			5-6
hydroxylated		6-8	53-55
diesters			19-21
Alcohols	R-OH	?	2-3
Hydrocarbons	C's and H's	49-51	1.5-3
Sterols	tetracyclic rings	7-8	
Resins		5-6	4-6
Free Acids	 $\overset{O}{R}\text{-}C\text{-}OH$	7-9	3-3.5
Lactides			2-3
Moisture, Minerals, and Inorganic Residue		0.5-3	0.5-1

[1] Kirk and Othmer, 1984; Bennet, 1975; Warth, 1956.

L.) and now is produced by high pressure hydrogenation or electrolytic reduction of D-glucose, or by catalytic hydrogenation of dextrose. It has a molecular weight of 182 daltons, consisting of a 6 carbon chain with 6 hydroxyl groups ($C_6H_{14}O_6$)(Appendix A). It is mainly found in the stable crystalline (γ-) form, with a melting point of 96°C. It is highly soluble in water (solubility of 234g/100g water at 25°C), but insoluble in most other organic solvents. It is stable and chemically unreactive. At equal RH'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.

1.3 Structure and Characterization of Edible Films

Structure and characterization of edible films have been used to explain the functional properties which they possess. Methods commonly used to determine microstructure are x-ray diffraction, scanning electron microscopy (SEM), and transmission electron microscopy (TEM).

Carnauba and candelilla wax films were found to have a high degree of orthorhombic packing, with side-to-side hydrocarbon chain packing. The three most common types of hydrocarbon packing are triclinic, orthorhombic, and hexagonal. Hexagonal packing is the least dense with the lowest melting point, while triclinic is the most dense and has the highest melting point. Candelilla wax had a higher relative intensity (RI) ratio (0.65), from x-ray diffraction, compared to carnauba wax (0.41). This value being stated as the RI at 3.74 Angstroms/RI at 4.13 Angstroms. As RI increases the amount of hexagonal packing decreases.

In SEM's of the top surface, that exposed to the environment during drying, neither had penetrating imperfections. Candelilla wax films were shown to be relatively smooth, while carnauba wax films possessed a 'hilly' appearance (Donhowe and Fennema, 1993).

Beeswax (BW) films are similar in composition to carnauba and candelilla wax. They were also found to be highly orthorhombically packed (RI being 0.34), with side-to-side hydrocarbon chain orientation. They were shown to be smooth and free of imperfections (Donhowe and Fennema, 1993; Kester and Fennema, 1989a). Greener and Fennema (1989) found similar morphology in a BW laminate film. Where the BW was formed onto the surface of a methylcellulose (MC) film. SEM showed a continuous and homogeneous MC and lipid layer present in cross sectional analysis of MC/lipid laminate films (Park *et al.*, 1996).

In MC and paraffin wax emulsion films wax droplets could be seen dispersed throughout the protein matrix. These droplets causing a 'hilly' appearance to the surface (Martin-Polo *et al.*, 1992; Debeaufort *et al.*, 1993).

Milk protein and fatty alcohol or fatty acid emulsion films showed the presence of crystalline platelets on the top surface (McHugh and Krochta, 1994b & d; Ho, 1992). The size and number irregular structures increased as chain length decreased and concentration of the lipid increased. More surface irregularities appeared in caseinate/fatty acid films compared to caseinate/fatty alcohol films, with lipids of equal carbon backbone length (Ho, 1992).

Whey protein isolate (WPI)/BW emulsion films were also reported as having discrete crystalline particles present (McHugh and Krochta, 1994d). Further studies showed that increased homogenization of the film forming solution resulted in a smaller lipid particle size and a more even distribution throughout the protein matrix (McHugh and Krochta, 1994b). Caseinate/BW showed the appearance of wax droplets dispersed uniformly throughout the protein matrix. Similar results were found for caseinate/carnauba wax films (Ho, 1992).

1.4 Intrinsic Bulk Properties

Intrinsic bulk properties are influenced by the molecular mass and distribution, and how the polymer chain is structured, either linear, branched, or crosslinked (Hernandez, 1997). Some important intrinsic bulk properties for edible films include the melt range (T_m) and the heat seal temperature range. With a higher melting temperature (T_m) a film or material generally possesses less low melting components, which can correspond to a stiffer less permeable film. This is a result from stronger packing and more secondary bonding, thus less chain mobility (Birley, Haworth, and Batchelor, 1992). Differential scanning calorimetry (DSC) is the general method used in testing thermal properties.

Donhowe and Fennema (1993) performed thermal studies on microcrystalline wax, BW, carnauba wax, and candelilla wax. They found that microcrystalline wax to have a broad melting range, with a T_m of 71.7°C. BW and candelilla wax were similar in that their melting points were 62.0 and 64.0°C, respectively, with each having a solid-solid (s-s) transition. Carnauba wax had a melting point of 81.7°C, with two s-s transitions.

1.5 Properties of Films Important for Food Applications

1.5.1 Barrier

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

The rate at which these gases or vapors pass through a polymer is known as it's permeation. There are three steps involved in the action of permeation, absorption of the gas or vapor 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:

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

where P = permeability coefficient, D = diffusion coefficient, and S = 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:

$$C_D = S_P \qquad (2)$$

where C_D = dissolved equilibrium concentration, and S_P = 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 was developed, based on Henry's law. This expression states:

$$C^* = C_D + C_H \qquad (3)$$

where C^* = total effective gas concentration, and C_H = 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:

$$J = -D\ (\delta C/\delta x) \qquad (4)$$

where J = flux, the rate of transfer per unit area, D = diffusion coefficient, and $\delta C/\delta x$ = 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:

$$\delta C/\delta t = D(\delta^2 C/\delta x^2) \qquad (5)$$

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

There are two basic processes for determining the permeation of the gas or vapor, the isostatic or quasi-isostatic method. In both cases you have the permeant flowing over one side of the film, permeating through the film and collected on the other side of the film. 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 quantitating sensor, while with the quasi-isostatic method the permeant is allowed to collect in the diffusion

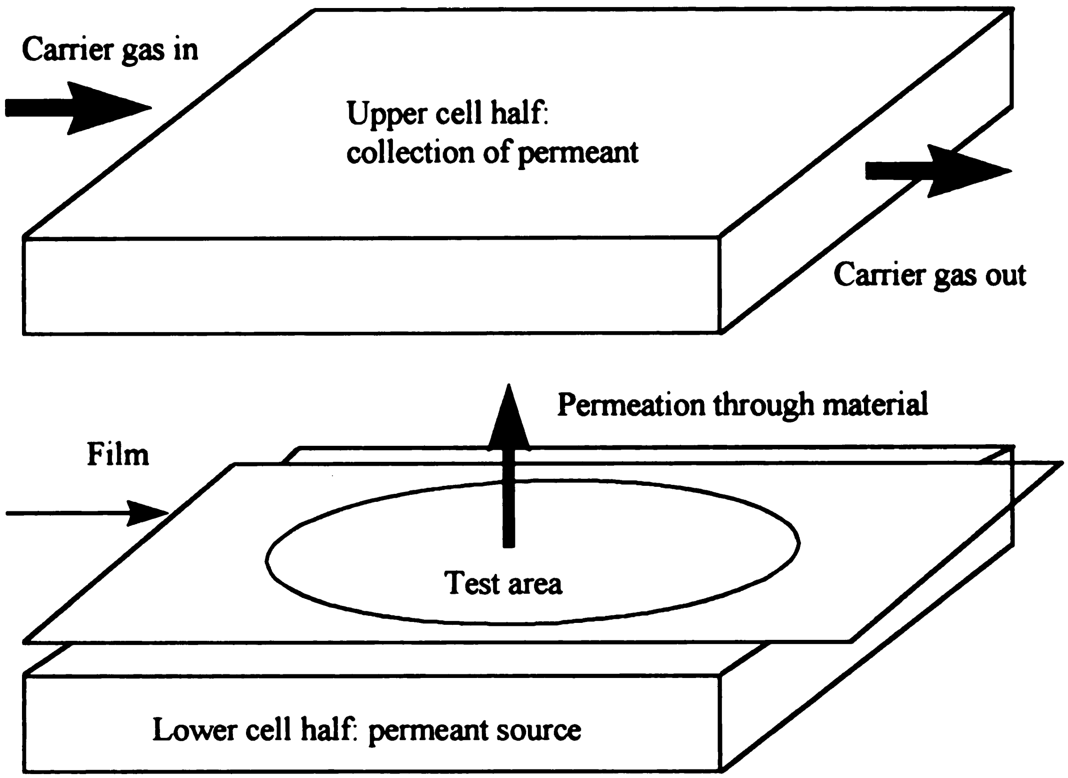


Figure 1. The Diffusion Cell for Permeability Testing

cell and then sampled over a period of time. The test is generally run until steady state is reached.

1.5.1.1 Water Vapor Permeability (WVP) of Edible Films

Lipid films have been found to provide a water barrier similar to that of high density polyethylene (HDPE), which is considered to be an excellent barrier to water. Protein films do not provide good barriers to water, due to their hydrophilic nature (Table 3).

Donhowe and Fennema (1993) studied the WVP of wax films and found candelilla wax films displayed the best water barrier properties, similar to HDPE. Microcrystalline and carnauba wax had WVP values almost double that of candelilla wax, with BW being almost three times larger. The higher WVP in microcrystalline wax was attributed to the absence of polar lipids, causing a less rigid structure. The larger concentration of polar compounds (fatty acids, fatty alcohols, and esters) in BW was attributed to it's higher WVP. Carnauba wax, while having a high concentration of polar compounds, possessed the highest melting point (less low melting fraction) providing it with less molecular mobility. The superior barrier properties of candelilla wax were found to be due to the low concentration of polar compounds, the large concentration of alkanes, and being the most orthorhombically packed of the waxes (Donhowe and Fennema, 1993).

Kester and Fennema (1989b) reported the water barrier properties of their films in terms of the resistance to water vapor transmission. This was calculated according to:

$$r = \Delta c / J \qquad (6)$$

Table 3. Water Vapor Permeability (WVP) of Selected Edible Films.

Film Type[1]	WVP x10^{14} (kg m/m^2 s Pa)	Testing Conditions	Reference
Carnauba Wax	0.033	21°C, 0%/85% RH	Donhowe and Fennema (1993)
Candelilla Wax	0.018	21°C, 0%/85%	"
Beeswax	0.058	21°C, 0%/85%	"
CZ(83)-G(17)	8.9-13.3	21°C, 0%/85%	Park and Chinnan (1995)
SPI(63)-G(37)	179-304	25°C, 0%/50%	Brandenburg *et al.* (1993)
WG(71.4)-G(28.6)	125.4	26°C, 50%/100%	Aydt *et al.* (1991)
NFDM(75)-G(25)	93.7	21°C, 0%/85%	Maynes and Krochta (1994)
WPI(50)-G(50)	7.5	25°C, 0%/77%	McHugh and Krochta (1994)
WPI(50)-S(50)	4.1	25°C, 0%/77%	"
WPI(62.5)-S(37.5)	3.0	25°C, 0%/77%	"
WPI(67)-G(33)	336.5	23°C, 55%/100%	Banerjee and Chen (1995)
WPC(67)-G(33)	295.5	23°C, 55%/100%	"
CC(67)-G(33)	219.6	23°C, 55%/100%	"
SC(67)-G(33)	358.2	23°C, 55%/100%	"
PC(67)-G(33)	349.0	23°C, 55%/100%	"
RC(50)-S(50)	57.4	37.8°C, 0%/90%	Chick (1996)
LAC(50)-S(50)	52.1	37.8°C, 0%/90%	"
LAC(50)-G(50)	68.6	37.8°C, 0%/90%	"
LAC(58)-S(42)	39.3	37.8°C, 0%/90%	"
WPI(67)-G(33)-CR(40)	38.2	25°C, 0%/100%	Shellhammer and Krochta (1997)
WPI(67)-G(33)-CN(40)	35.9	25°C, 0%/100%	"
WPI(67)-G(33)-BW(40)	12.5	25°C, 0%/100%	"

[1] CZ - corn zein, SPI- soy protein isolate, WG - wheat gluten, NFDM - non fat dry milk, WPI - whey protien isolate, WPC - whey protein concentrate, CC - calcium caseinate, SC - sodium caseinate, PC - potassium caseinate, RC - rennet casein, LAC - lactic acid casein, G - glycerol, S - sorbitol, CR - carnauba wax, CN - candelilla wax, BW - beeswax. Numbers in parentheses are % of film composition.

where r = resistance to vapor or gas transmission, Δc = driving force for vapor or gas transmission in terms of concentration gradient across the film, J = steady state flux of the vapor or gas.

BW films formed on Whatman No. 50 filter paper BW provided resistance to water transmission similar to that of PET. These properties were attributed to the smooth, defect free surface, and it's orthorhombic packing. Hydrocarbon chains present on the surface mask the polar groups of wax esters. Esters will not spread on the surface of water (Kester and Fennema, 1989b).

In protein films it was found that formation of films at a pH away from the isoelectric point (pI) provided them with better water barrier properties (Stuchell and Krochta, 1994; Gennadios *et al.*, 1993a). In preformed films, however, it was found that water barrier properties were enhanced by adjusting the films pH to the pI of the protein (Avena-Bustillos and Krochta, 1993). This allowed for more protein-protein interactions.

The type and the concentration of plasticizer has also been found to effect the water barrier properties of films (Chick, 1996; McHugh *et al.*, 1994a; Gennadios *et al.*, 1993a). As plasticizer concentration decreased, an increase in overall protein concentration, a decrease in WVP has been witnessed. Sorbitol was found to provide a film with better water barrier properties then either glycerol or polyethylene glycol. This was attributed in part to the ability of glycerol to absorb more water than sorbitol (Sicard and Leroy, 1983). Protein concentration was shown not to effect water barrier properties in casein films (Chick, 1996). The high plasticizer content in these films, 42% minimum, most likely being the main factor in these results.

Different variations of the same protein have been found to effect water barrier properties of protein films (Chick, 1996; Banerjee and Chen, 1995; Avena-Bustillos and Krochta, 1993). Whey protein concentrate (WPC) films promoted a better water barrier than WPI films (Banerjee and Chen, 1995). A higher residual fat content present in WPC was cited as the main reason for the better properties. Calcium caseinate (CC) films were shown to provide a better water barrier than other caseinate films (Banerjee and Chen, 1995; Avena-Bustillos and Krochta, 1993). Stronger ionic crosslinking was present in CC films, decreasing segmental rotation. Chick (1996) found no difference in water barrier properties of lactic acid casein and rennet casein films. The lactic acid casein in this study contained a higher protein and fat content than the rennet casein, 87.3, 1.2% and 80.6, 0.5% respectively. Rennet casein, however, does not have the hydrophilic macropeptide (in κ-casein) associated with it, and contained a higher ash content, 7.8% compared to 1.8%. Calcium, a portion of the overall ash content, present in rennet casein films could have provided for more calcium crosslinking.

In wax emulsion films, as lipid concentration was increased in emulsion films the water barrier properties improved (Kim and Ustunol, 1997; Baker, 1996; McHugh and Krochta, 1994d; Avena-Bustillos and Krochta, 1993; Ho, 1992). This improvement would continue until a critical lipid content was reached, at which point no further reduction in WVP would occur.

As the chain length of the lipid increases the water barrier properties were improved in emulsion films, due to more chain mobility associated with the shorter carbon chains (McHugh and Krochta, 1994d; Kamper and Fennema, 1984). Fatty alcohol emulsion films provide poorer water barriers than fatty acid emulsion films of the same chain length. The polar hydroxyl group in the fatty alcohol provided more interaction with water. BW emulsion films were found to provide a better water barrier than stearic acid emulsion films (Avena-Bustillos and Krochta, 1993). BW emulsion films also provided a better water barrier than candelilla wax emulsion films, which in turn were found to be better than carnauba wax emulsion films (Shellhammer and Krochta, 1997; Gontard *et al.*, 1994; Ho, 1992). In emulsion films, carnauba wax was found to be difficult to mix into solution.

Changes in RH have been found to effect the water barrier properties of edible films. Fennema, Donhowe, and Kester (1994) observed no change in the water resistance of BW films at 0 and 50% RH (100% RH on the other side of the film), while a decrease in resistance occurred at 65 and 80% RH (100% RH on the other side of the film). These results coincided with moisture sorption results for BW.

McHugh *et al.* (1994a) found similar results for WPI films made with sorbitol or glycerol. An exponential increase in WVP was observed at about 30% RH, with a more pronounced RH effect on films containing glycerol. In SC films an exponential increase in WVP was witnessed at around a 65% RH (McHugh, Avena-Bustillos, and Krochta, 1993).

WVP of cellophane films dipped in paraffin wax were equivalent at 22 and 44% RH, then increased at 57% RH (Martin-Polo, Manguin, and Voilley, 1992). In WPI-BW emulsion films an exponential increase was observed at around 50% RH, this same increase was seen at around 30% RH in films without BW (McHugh and Krochta, 1994d). BW was also seen to decrease the effect of RH on WVP. Kim and Ustunol (1997) observed WVP's approximately ten times greater at 80% RH compared to that at 50% in films comprised of WPC or WPI, with or without candelilla wax.

1.5.1.2 Oxygen Permeability (OP) of Edible Films

Lipid films have been found to be rather poor oxygen barriers. Most of which falling in the permeability range of high density polyethylene (HDPE) to polyethylene terephthalate (PET), both considered poor oxygen barriers (Donhowe and Fennema, 1993; Kester and Fennema, 1989a). Protein films, however, have generally possessed good oxygen barrier properties, comparable to that of nylon (Table 4).

Donhowe and Fennema (1993) found that candelilla and carnauba wax films displayed much better oxygen barrier properties than microcrystalline wax or BW films. Microcrystalline wax films were found to have the highest OP of the waxes. They attributed this to the microscopic sized crystals creating intercrystalline paths for oxygen permeation. They also suggest the presence of irregular crystal sizes and lattice distortions, leading to these intercrystalline pathways. Microcrystalline wax also contains some distillate oil, creating a less rigid structure.

The OP of BW films were found to be two times higher than HDPE. The presence of unsaturated hydrocarbons was cited as the reason for higher permeability. Kester and Fennema (1989a), however, found BW films to be a much better O_2 barrier than HDPE. The difference being that these films were formed on Whatman No. 50 paper, which could have aided in their barrier properties. Candelilla wax, being the most orthorhombically packed created it's better oxygen barrier film properties. Carnauba wax films had similar OP properties to candelilla wax, with it's smaller low melting point fraction making up for

Table 4. Oxygen Permeability (OP) of Selected Edible Films.

Film Type[1]	**OP x 10 (m^3 m/m^2 s Pa)**	**Testing Conditions**	**Reference**
Carnauba Wax	180	21°C, 0% RH	Donhowe and Fennema (1993)
Candelilla Wax	200	21°C, 0%	"
Beeswax	1080	21°C, 0%	"
CZ(83)-G(17)	15.0-51.9	21°C, 0%	Park and Chinnan (1995)
SPI(63)-G(37)	5.5	25°C, 0%	Brandenburg *et al.* (1993)
WG(71.4)-G(28.6)	4.4	23°C, 0%	Gennadios (1993)
WPI(70)-G(30)	88.0	23°C, 0%	McHugh and Krochta (1994)
WPI(70)-S(30)	5.0	23°C, 0%	"
WPI(50)-S(50)	9.6	23°C, 0%	"
RC(50)-S(50)	1.18	23°C, 0%	Chick (1996)
LAC(50)-S(50)	0.85	23°C, 0%	"
LAC(50)-G(50)	2.52	23°C, 0%	"
LAC(58)-S(42)	0.94	23°C, 0%	"
WPI(55.6)-S(28.5)-BW(16)	13.4	23°C, 0%	McHugh and Krochta (1994)

[1] CZ - corn zein, SPI - soy protein isolate, WG - wheat gluten, WPI - whey protien isolate, RC - rennet casein, LAC - lactic acid casein, G - glycerol, S - sorbitol, BW - beeswax. Numbers in parentheses are % of film composition.

it being less orthorhombically packed than candelilla wax.

WG films were found to provide a better oxygen barrier than corn zein (CZ) films. This was attributed to a high α-helical content in CZ films (Gennadios, Weller, and Testin, 1993c).). WG properties were derived from it's tight and complex structure formed by extensive covalent and non-covalent bonding, with inter- and intramolecular disulfide bonding (Gennadios *et al.*, 1993c).

Similar to WVP an increasing pH above the pI was found to enhance the oxygen barrier properties (Rayas, 1996; Brandenburg *et al.*, 1993). The effect that plasticizer type and concentration have on the OP of WPI-based films was studied by McHugh and Krochta (1994c). They found that films containing sorbitol will provide a better oxygen barrier than films containing glycerol, at equal concentrations. They also showed that as plasticizer concentration increased OP increased.

OP was found to be between EVOH and Nylon 6 in films made with lactic acid or rennet casein (Chick, 1996). Protein concentration was found to have no significant effect on OP. Protein or plasticizer type also showed no significant effect on OP, except with rennet casein films plasticized with glycerol, which had higher OP values.

In emulsion films made with WG and AM there was no significant difference in OP's compared to films without lipid (Gennadios, Weller, and Testin, 1993d). However, lipid content was below 3%. Kim and Ustunol (1997) reported a significant decrease in OP in emulsion films made from WPI or WPC and candelilla wax compared to films without wax (wax content greater than 2%). They also reported no significant differences in OP among films containing 2, 4, or 8% wax.

The effect of RH on OP was studied by Rayas (1996). No significant effect was observed until about 30% RH, here a significant increase in OP was seen. This data correlated fairly closely with moisture sorption isotherms of WG films. This same effect was seen in WPI films (McHugh and Krochta, 1994c). WPI-glycerol films witnessed no significant RH effect until 30 or 40% when an exponential increase in OP was observed, with maximal OP occurring at about 75% RH. WPI-sorbitol films were similar with the exponential increase occurring at around 50% RH and the maximum OP being witnessed also at 75%. These results were similar to the effects of RH on WVP properties of these WPI films.

Rico-Pena and Torres (1990) observed no RH effect in the 0 to 57% range in MC-palmitic acid films. However, a doubling of OP was observed at 79% RH. These results also coincided with moisture sorption isotherms. Hagenmaier and Baker (1996) reported OP values of 60% gelatin and 40% candelilla wax-oleic acid emulsion films to be equivalent at 40 and 55% RH, with and increase at 75%. In films comprised of 20% gelatin and 80% candelilla wax-oleic acid OP's at 40 and 55% were equivalent, however, a decrease was observed at 75%.

Hernandez (1994) and Gavara and Hernandez (1994) studied the effects of RH on nylon 6 and amorphous nylon films tested at 23°C. In amporphous nylon they observed a sharp decrease in OP from 0 to 20% RH reaching an asymptotic value after that. This was explained by the combination of an initial increase in diffusion from the 0 to 5% RH region followed by a slow increase in diffusion and a sharp decrease in oxygen solubility in the 0 to 20% RH region which then leveled off. The sharp decrease in solubility was explained by the competition for chemi-active sites between water and oxygen, with the interaction between water and amophous nylon being stronger in this region (Hernandez, 1994). In semicrystalline nylon 6 films OP decreased from 0% RH to a minimum reached in the range of 30 to 50% RH followed by an increasing OP with an increasing RH (Hernandez, 1994; Gavara and Hernandez, 1994). Diffusion was similar to that of amorphous nylon (Gavara and Hernandez, 1994), while solubility acted similar to it's OP, with a minimum in the 30 to 40% RH range (Hernandez, 1994). Here, formation of water clusters may have disrupted the polymer crystalline regions instead of filling void regions as with the amorphous nylon (Hernandez, 1994).

1.5.2 Mechanical Properties of Edible Films

The mechanical properties a polymer possesses can be determined by it's stress-strain tensile characteristics. Stress is measured as the force/area, while strain is the dimensionless fractional length increase (Hernandez, 1997). The ultimate tensile strength (TS) is the maximum tensile stress a material can sustain. Ultimate elongation (E%) is the strain at which the sample ruptures. Strength increases with an increase in molecular mass and an increase in intermolecular forces, but decreases with the presence of plasticizers. Generally, the TS and E% of edible films have not been as high as synthetic polymers (Table 5).

TS was found to increase as pH increased (Brandenburg *et al.*, 1993; Gennadios *et al.*, 1993a). The E% was also found to peak at higher pH's, however, differences were not significant compared to other pH values. The use of crosslinking agents was found to increase the tensile strength, with no effect to the E% (Rayas, 1997; Motoki *et al.*, 1987).

Table 5. Mechanical Properties of Selected Edible Films.

Film Type[1]	TS (MPa)	E (%)	Reference
SPI(63)-G(37)	3.1-5.2	66.5-90.3	Brandenburg *et al.* (1993)
WG(71.4)-G(28.6)	1.8	25	Aydt *et al.* (1991)
NFDM(75)-G(25)	9.1	5.2	Maynes and Krochta (199)
WPI(70)-G(30)	13.9	30.8	McHugh and Krochta (1994)
WPI(70)-S(30)	14.0	1.6	"
WPI(50)-S(50)	14.7	8.7	"
WPC(67)-G(33)	3.4	20.8	Banerjee and Chen (1995)
WPI(67)-G(33)	5.9	22.7	"
CC(67)-G(33)	4.3	1.5	"
SC(67)-G(33)	3.0	29.9	"
PC(67)-G(33)	3.0	42.8	"
RC(50)-S(50)	9.5	77.6	Chick (1996)
LAC(50)-S(50)	7.5	156.0	"
LAC(50)-G(50)	1.2	253.6	"
LAC(58)-S(42)	11.6	150.6	"
WPI(67)-G(33)-CR(40)	≅ 23	≅ 1.5	Shellhammer and Krochta (1997)
WPI(67)-G(33)-CN(40)	≅ 16	≅ 1.0	"
WPI(67)-G(33)-BW(40)	≅ 18	≅ 2.0	"

[1] CZ - corn zein, SPI - soy protein isolate, WG - wheat gluten, WPI - whey protien isolate, RC - rennet casein, LAC - lactic acid casein, G - glycerol, S - sorbitol. Numbers in parentheses are % of film composition.

With an increase in plasticizer content (a decrease in protein content) a decrease in TS is observed while E% increased (Maynes and Krochta, 1994; Stuchell and Krochta, 1994).

Banjeree and Chen (1995) demonstrated that WPI produced stronger films than WPC. E% was found to be similar between the WPI and WPC films. CC films produced stronger films than SC or PC. However, the reverse of this was true pertaining to E%. Due to the stronger ionic crosslinking in CC, creating a less extensible film.

Gennadios *et al.* (1993b) found TS to decrease as RH increased in both CZ and WG films. RH was shown to have more of an effect on WG films, due to WG being more hydrophilic than CZ. Isotherms of CZ and WG films showed this to be true (Gennadios and Weller, 1994).

In emulsion films the addition of lipids to protein films has produced mixed results in terms of the films TS. However, E% has either remained unchanged or decreased with the addition of lipids (Shellhammer and Krochta, 1997; Kim and Ustunol, 1996; Banerjee and Chen, 1995; Debeaufort and Voilley, 1995; Gennadios *et al.*, 1993).

Shellhammer and Krochta (1997) found a fairly linear decrease in TS with the addition of wax from 10 to 90%, in films comprised of WPI and candelilla wax, carnauba wax, or BW. Films containing carnauba wax were always found to be the strongest, while candelilla wax always produced the weakest films. The addition of BW did not have much of an effect on E%, while the addition of candelilla and carnauba wax produced a slight reduction in E%. Kim and Ustunol (1996) found a significant increase in TS in WPI-candelilla wax emulsion films as wax content increased, from 0 to 8%. In WPC-candelilla wax emulsion films a significant increase in TS was observed with the addition of wax, but did not increase with increasing wax content, from 2 to 8%. The addition of candelilla wax to WPC films was found to decrease E%, but that decrease remained unchanged with an increasing wax content, from 2 to 8%. In WPI-candelilla wax emulsion films a significant decrease in E% was not observed until the addition of 4% wax.

2.0 MATERIALS AND METHODS

2.1 Film Development

The primary film components were lactic acid casein powder, carnauba or candelilla wax, and sorbitol. Lactic acid casein powder (Alacid 710, 30 mesh),which will be referred to as lactic acid casein, was obtained from New Zealand Milk Products (N. America) Inc., (Santa Rosa, CA)(Table 6). Carnauba and candelilla wax were obtained from Strahl and Pitsch Inc. (West Babylon, NY). Sorbitol was purchased from Sigma Chemical Co., (St. Louis, MO).

Figure 2 shows a schematic diagram of the film forming process. Emulsion solutions, 150 g final weight, were prepared by first mixing lactic acid casein (5% w/w) with sorbitol (3.5, 4, 4.5, or 5% w/w), distilled water, and 1M NaOH (to adjust pH to 10.0). Solutions were heated and stirred, using the "Magna-4" magnetic stirrer and hot plate, model 4820-4 (Cole-Parmer, Chicago, IL), for 30 minutes to a final temperature of 65.5 ± 2.5°C, and then held at that temperature for 15 minutes. This process solublized the lactic acid casein and sorbitol in accordance with that developed by Chick (1996).

Solutions were then heated to the melting temperature of the wax, 75 ± 2°C for solutions where no wax or candelilla wax were to be added, and 85 ± 2°C for solutions where carnauba wax was to be added. Melted wax (0.5, 1, or 1.5% w/w) was then added to the solutions, with the total primary film components of the final solutions (casein, sorbitol, and wax) being maintained at 10% w/w (Table 7).

This was followed by homogenization for 1 min with a Polytron PT 10/35 (Brinkmann Instruments, Switzerland) using a PTA 20 TS generator (homogenizing

Table 6. Composition of Lactic Acid Casein Powder[1]

	% found in protein powder
Protein (N x 6.38)	87.3
Ash	1.8
Moisture	9.6
Fat	1.2
Lactose	0.1

[1] Values based on company specifications with pH (5% at 20°C) being 4.6 (New Zealand Milk Products (N. America) Inc.)

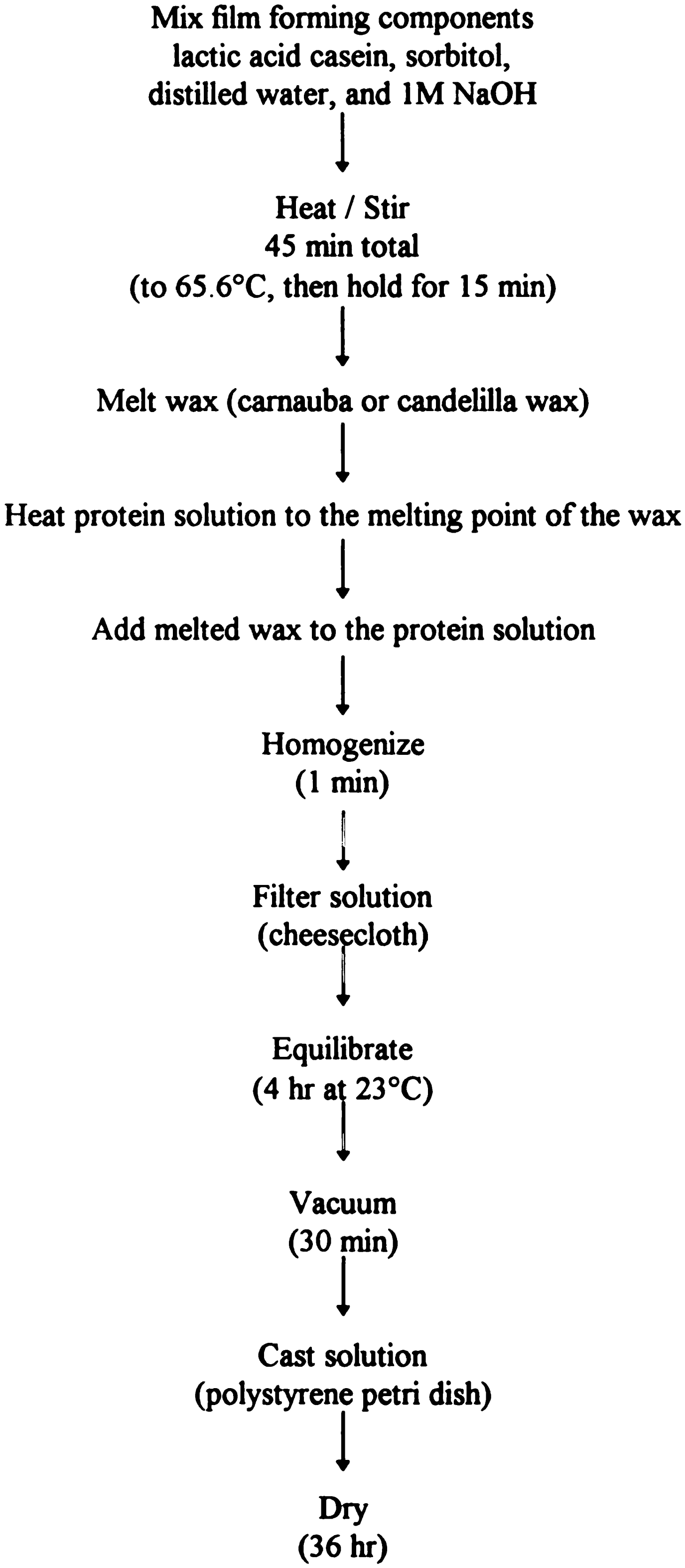


Figure 2. Schematic Diagram of the Film Forming Process

Table 7. Composition of Lactic Acid Casein-Based Edible Films

	Primary Film Components					
	Lactic Acid Casein Powder		**Sorbitol**		**Wax (Carnauba or Candelilla)**	
Treatment	**% w/w aqueous solution**	**% total primary components**	**% w/w aqueous solution**	**% total primary components**	**% w/w aqueous solution**	**% total primary components**
1	5	50	5.0	50	0	0
2	5	50	4.5	45	0.5	5
3	5	50	4.0	40	1.0	10
4	5	50	3.5	35	1.5	15

head) and a PCU 11 power control unit at setting 5 (settings ranging from 0 to10). This was performed in order to produce more uniform was particles within the film structure, as was shown by McHugh and Krochta (1994b).

Solutions were maintained at 23 ± 2.0°C for 4 hours to allow any foaming created during the mixing/homogenization process to settle. Next, samples were filtered through 1 layer of cheesecloth. A vacuum was applied to the solutions for 30 minutes, using a hydrometric vacuum system, to remove any residual air in the solution. The film forming solution (19 ± 2mL) was then cast on to a 150 x 15mm polystyrene petri plate. Casting was performed using these petri plates as compared to teflon coated pans previously used (Chick, 1996), in an attempt to better control thickness. These petri plates provided a smaller and more level casting surface than that provided by the larger teflon coated pans. Drying was carried out at 23 ± 2°C and 50 ± 5% RH for 36 ± 5 hours, at which point films were able to be peeled intact from the casting surface. Previously, films were dried in an oven to reduce drying times (Chick, 1996), however, again to better control thickness films were dried in room conditions on a smooth level surface. The oven racks were found not to be completely level. Once peeled from the casting surface, films were kept at 23 ± 2°C until testing was performed.

2.2 Film Microstructure

Microstructure of film samples were determined using a scanning electron microscope, JOEL 6400V SEM (Japan Electron Optics Limited, Japan). Samples were prepared using standard techniques, mounted on aluminum stubs and sputter coated with gold (20nm). Samples were observed using an accelerating voltage of 10 to 15kV. SEM pictures were taken of the films top and bottom surface, the top surface being that exposed to the air when cast, and the cross section. SEM pictures of heat sealed films were taken of the cross section of the seal area, using an accelerating voltage of 13kV. For surface analysis a magnification of 500X was used, while for cross sectional analysis magnification ranged between 350 to 500X, fitting the entire cross section into the picture. A representative sample of each film type was used for SEM studies.

2.3 Thermal Characteristics

Thermal characteristics were determined by differential scanning calorimetry (DSC), DSC 2920 Modulated DSC (TA Instruments). Samples, 6 to 12mg, were sealed in a standard aluminum dish, with a sealed empty aluminum dish used as the reference sample for testing. Tests were conducted from -25 to 150°C with an increase in temperature of 10°C/min. Lactic acid casein, carnauba wax, candelilla wax, and sorbitol, and film samples of lactic acid casein (50%)-sorbitol (50%), lactic acid casein (50%)-sorbitol (35%)-carnauba wax (15%), and lactic acid casein (50%)-sorbitol (35%)-candelilla wax (15%)(the composition is reported on a primary film component basis, unless otherwise specified) all tests were carried out in triplicate.

2.4 Moisture Sorption Isotherm

A moisture sorption isotherm curve was determined for lactic acid casein (50%)-sorbitol (50%) film. Five to six grams of film, cut into small pieces, were placed into aluminum dishes then allowed to reach equilibrium (when weight gain of sample ceased) at 23°C and RH's of 9.1, 34.8, 46.3, 54.0, 75.5, and 80.8%. Proper RH conditions were obtained by use of salt solutions placed in sealed 5 gallon HDPE buckets (Table 8). Once equilibrium was established, when weight gain ceased, moisture content was determined by drying film samples in a vacuum oven (Precision Scientific model 524) at 100°C for 24 hours (Banerjee and Chen, 1995), according to the equation:

$$MC = [1 - (wt_f)/(wt_i)] \times 100 \qquad (7)$$

where MC = percent moisture in the film sample, wt_f = final weight of the film sample, wt_i = initial weight of the film sample. MC values are reported as the average of 3 values at each RH condition.

The experimented moisture sorption isotherm values were fit by the Guggenheim-Anderson-DeBoer model (GAB):

$$EMC = (W_m\, C\, k\, a_w)/[(1- k\, a_w)(1- k\, a_w + C\, k\, a_w)] \qquad (8)$$

where EMC = equilibrium moisture content on a dry basis, W_m = monolayer in the B.E.T. theory, C = Guggenheim constant, k = factor correcting properties of the multilayer molecules with respect to bulk liquid, a_w = water activity (Bizot, 1983). Equation (8) can then be transformed to:

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

where:

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

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

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

where a quadratic equation is performed on experimental values to find α, β, and γ.

Table 8. Saturated Salt Solutions Used to Create RH Conditions in Moisture Sorption Isotherm Study

Sat. Salt Solution	**Formula**	**RH% (23°C)**
Lithium Chloride	$LiCl\ H_2O$	9.1
Magnesium Chloride	$MgCl_2\ 6H_2O$	34.8
Potassium Carbonate	$K_2CO_3\ 2H_2O$	46.3
Magnesium Nitrate	$Mg\ (NO_3)_2\ 6H_2O$	54.0
Sodium Chloride	$NaCl$	75.5
Ammonium Sulfate	$(NH_4)_2SO_4$	80.8

2.5 Barrier Properties

2.5.1 WVP

Water vapor transmission rate (WVTR) was tested at 37.8 ± 0.5°C and at 50 ± 3 and 70 ± 3% RH. according to ASTM standard F 1249-90, by using the Permatran-W 3/31 (MoCon Inc., Minneapolis, MN). The testing surface area of film samples were reduced from 50 to 5 cm^2 with the use of a foil backing, to stay within sensor range. Samples were conditioned for 6 hours at the RH conditions before testing was conducted. The WVTR was measured until steady state was reached. Steady state occurs when permeation of the gas or vapor is constant. Calibration of the system was executed with 0.025 mm (1 mil) thick standard polyester (PET) film. WVP was then calculated according to:

$$\text{WVP} = \text{WVTR} \times \ell / \Delta p \qquad (13)$$

where ℓ = thickness of film, Δp = pressure differential acting on the film. WVP values were the average of at least three film samples produced from a different film forming solution batch.

2.5.2 OP

Oxygen transmission rate (OTR) was evaluated according to ASTM standard D-3985-81, using a modified Oxtran 200 (MoCon Inc., Minneapolis, MN)(Figure 3)(Gavara and Hernandez, 1994). Tests were performed at 23 ± 0.5°C and 0, 20, 35, 50, and 70% RH. Humidity of the gas streams was monitored using wide-range hygrosensors (10-90 ± 2.0% RH) with a Hydrodynamics digital hygrometer indicator and controller, model 3088 (Newport Scientific, Inc., Jessup, MD). Only film samples with no wax and 15% wax

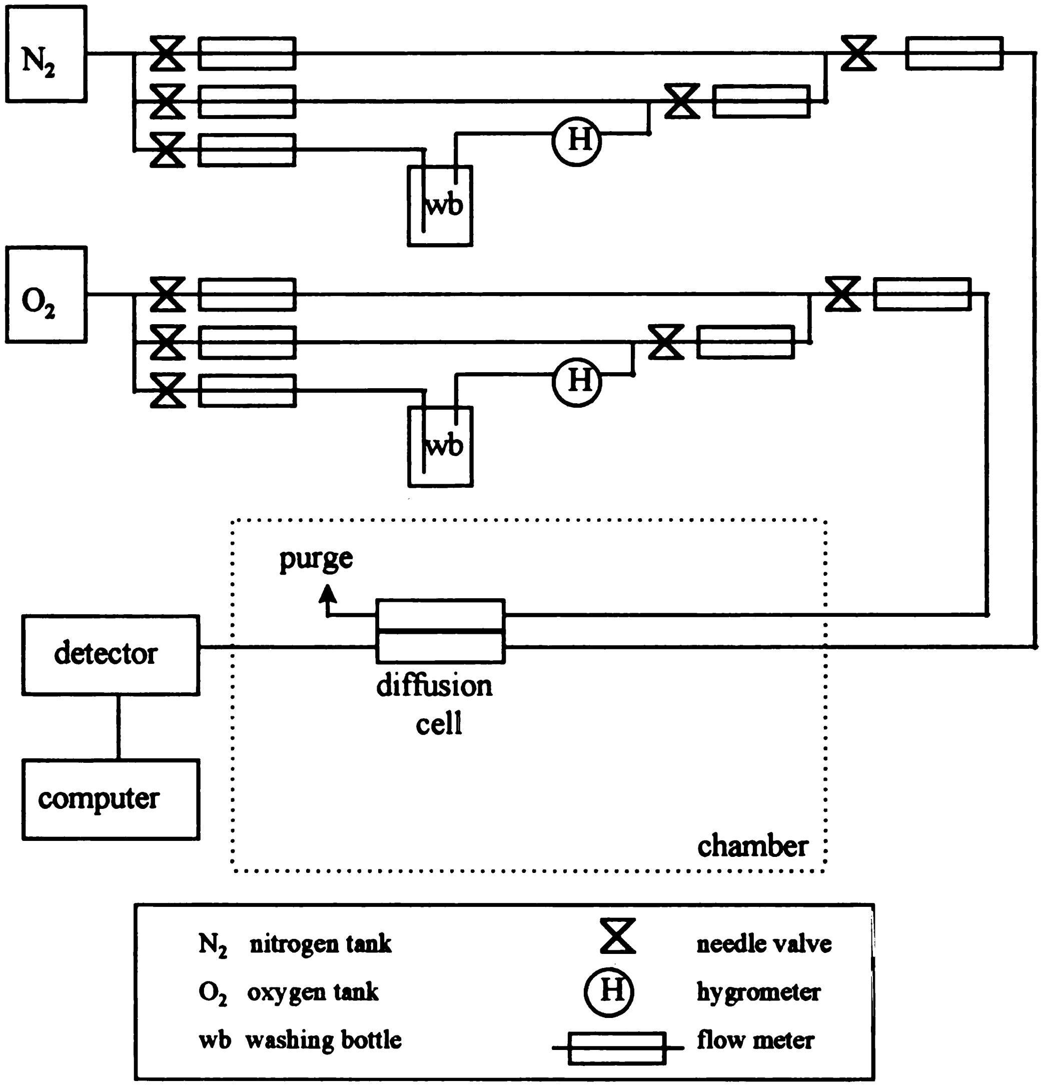


Figure 3. Modified Oxtran 200 Schematic

were evaluated at RH's above 0%. Samples with 15% wax content, instead of 5 and 10%, were chosen because they possessed the better water barrier properties.

A 50 cm^2 diffusion cell was used, with compressed air (1 atm) being the permeant and nitrogen the carrier gas. Samples were conditioned for at least 4 hours at the testing conditions before testing was conducted. The flow was measured until steady state was reached. Calibration of the system at 23 °C and 0% RH was executed with 0.025 mm (1 mil) standard PET film. This same PET film was also tested at all the elevated RH conditions. OP was then calculated according to:

$$OP = OTR \times \ell / \Delta p \qquad (14)$$

where ℓ = thickness of film, Δp = pressure differential acting on the film. OP values were the average of at least three film samples, each produced from a different film forming solution batch.

2.6 Mechanical Properties

2.6.1 Strength and Elongation

Tensile strength (TS) and elongation were tested according to ASTM standard D-882-3, using the Instron Universal Testing Machine model 4201 (Instron Corp., Canton, MA). TS and elongation tests were performed at 23 ± 2°C and either 50 or 75 ± 5% RH. Temperature conditions were maintained by the use of a conditioned room at 23°C and 50% RH, while the 75% RH was maintained by the use of a saturated salt solution (NaCl) in a sealed bucket HDPE stored in the conditioned room. Samples stored at 75% RH were tested at room conditions, however, the exposure time to these conditions was kept at an absolute minimum, the time needed to measure thickness and test the sample. Heat seal strength was tested at 23°C and 50% RH. Sample sizes for all tests were 2.54 cm wide and at least 8 cm long, with a jaw separation of 5.08 cm and a jaw speed of 50.8 cm/min (20 in./min). TS and elongation were calculated according to:

$$TS = ld/(\ell \times w) \quad (15)$$

where ld = load (lbs), ℓ = material thickness, w = material width.

$$\text{Elongation} = (\Delta L / L_i) \times 100 \quad (16)$$

where ΔL = change in length, L_i = original length.

All mechanical property values reported are the average of at least three film samples tested, each from a different film forming solution batch. The values of each individual film sample in each film forming solution batch was an average of at least two film samples from that batch.

2.6.2 Heat Sealing

Heat sealing of film samples was accomplished using a heat sealer, model 12ASL (Sencorp Systems, Inc., Hyannis, MA). Two strips of film, 2.54 cm wide and approximately 5.08 cm long, were cut, measured for thickness, then sealed widthwise. The seal area was approximately 2.54 cm x 1.5 cm. Seal temperatures ranged from 93 to 121°C, based on DSC and preliminary results. Dwell time was either 2 or 4 seconds, with a seal pressure of 40 or 60 psi. Seal strength was derived from the same test procedure and calculations as TS (equation 15). The seal was located midway between the jaw grips when tested. Heat seal testing was performed on film samples with no wax and 15% wax.

2.7 Thickness

Thickness was measured using a micrometer, TMI model 549M micrometer (Testing Machines Inc.; Amittyville, NY). For water and oxygen barrier testing thickness was the average of 5 measurements taken over the testing surface. Average thickness for WVP and OP were 0.104 ± 0.007 mm and 0.111 ± 0.009 mm respectively. For TS and elongation thickness was the average of three measurements taken over the length the test specimen. For heat seal strength testing thickness was the average measurement taken from each film layer in the area that the seal was formed in.

2.8 Statistical Analysis

Statistical analysis and appropriate comparisons as related to the effect of wax type, wax content, and the effect of RH on WVP, OP, TS, elongation, and the effect of sealing parameters (time, pressure, and temperature) on seal strength were made using Sigma Stat 1.0 (Jandel Corp., San Rafael, CA) performing multiple comparisons with the Student-Newman-Keuls method for multiple comparisons or analysis on ranks when equal variance was not observed. A randomized block design was used for all analyses.

3.0 RESULTS AND DISCUSSION

3.1 Film Development

Films formed without wax were smooth, transparent in appearance, and flexible. Films made with wax were smooth and flexible, however, they showed a hazy or opaque appearance. Wax could not be incorporated above 15%, at a protein powder concentration of 50%, because higher wax contents produced films that could not be separated from the casting surface. These results are similar to those found by Ho (1992). Carnauba wax was found to be more difficult to mix into solution than candelilla wax. This is shown by a non-consistent coloring in films made with carnauba wax, versus a consistent coloring in films with candelilla wax. This most likely occurs due to the higher melting point of carnauba wax, causing it to solidify more rapidly upon cooling. Homogenization was performed at room temperature, causing the quicker cooling of carnauba wax. Similar results were found in films produced by Gontard *et al.* (1994).

3.2 Microstructure

SEM's of the bottom surface, adjacent to the casting surface when drying, of films with no wax, 15% carnauba wax, and 15% candelilla wax showed no differences in appearance (Figures 4 and 5). In films without wax the top surface, open to the environment when drying, appeared to be similar to that of the bottom surface (Figure 6). Films without wax appeared to be smooth and defect free, except for occasional tiny air bubbles appearing on the surface.

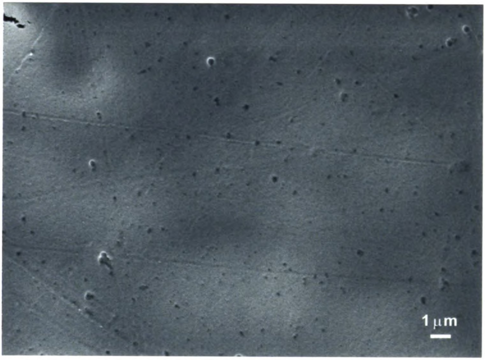


Figure 4. SEM of the Bottom Surface of a Lactic Acid Casein (50%)-Sorbitol (50%) Edible Film

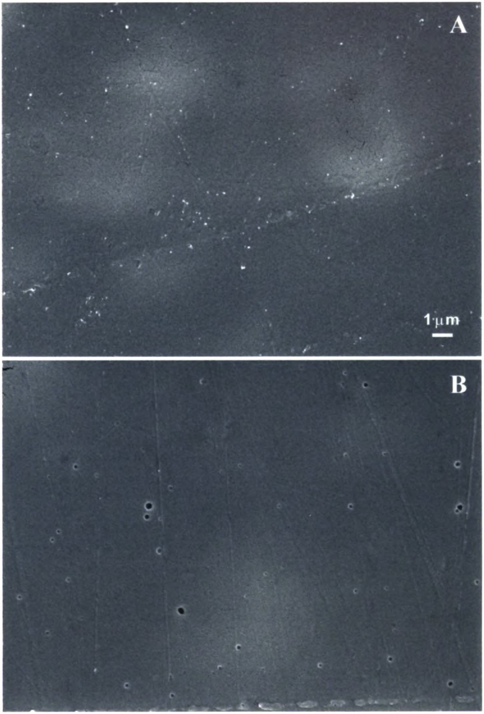


Figure 5. SEM of the Bottom Surface of a Lactic Acid Casein (50%)-Sorbitol (35%)-Carnauba Wax (15%)(top) and a Lactic Acid Casein (50%)-Sorbitol (35%)-Candelilla Wax (15%)(bottom) Edible Film

Figure 6. SEM of the Top Surface of a Lactic Acid Casein (50%)-Sorbitol (50%) Edible Film

Top surface microstructure of films containing wax, 5 or 15% carnauba wax and 5 or 15% candelilla wax, showed the appearance of wax droplets dispersed throughout the film giving the films a 'hilly appearance' (Figures 7 and 8). These results correspond well with those found by Ho (1992) in caseinate-BW and caseinate-carnauba wax films. Wax droplet size appeared to be fairly consistent due to the homogenization process. McHugh and Krochta (1994b) showed this to occur in WPI-BW-sorbitol emulsion films. When comparing SEM's of the top surface to those of the bottom surface in films containing wax, a separation of the wax, with the wax being mainly dispersed at or near the top surface is seen. No significant morphological differences could be seen among the surface of films containing carnauba wax or candelilla wax. The increased wax content can be observed when comparing SEM's of films containing 5% wax to those with 15% wax, coverage of the films surface with wax is more complete in films containing 15% wax (Figures 7 and 8).

Cross sectional analysis showed that the films were continuous and homogeneous (Figures 9, 10, and 11). No defects, like air bubbles, were found to go through the entire thickness of the films. In films without wax a layering structure is present (Figure 9). One layer shows up at the bottom surface, and is what we believe to be unsolubilized sorbitol (Figure 9). This layer also appears in films containing wax, most prevalent in those containing candelilla wax (Figure 11). This is assumed to be sorbitol because it's relative thickness appeared to decrease as the sorbitol content decreased, protein content remained constant. A thin layer is also seen near the top surface of films without wax. This is believed to be a drying layer, because of the increase in cracking evident (Figure 9). This

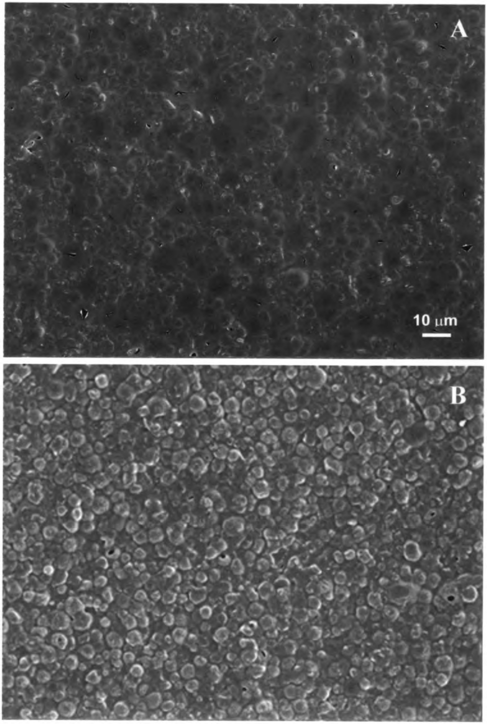


Figure 7. SEM of the Top Surface of a Lactic Acid Casein (50%)-Sorbitol (45%)-Carnauba Wax (5%)(top) and a Lactic Acid Casein (50%)-Sorbitol (35%)-Carnauba Wax (15%)(bottom) Edible Film

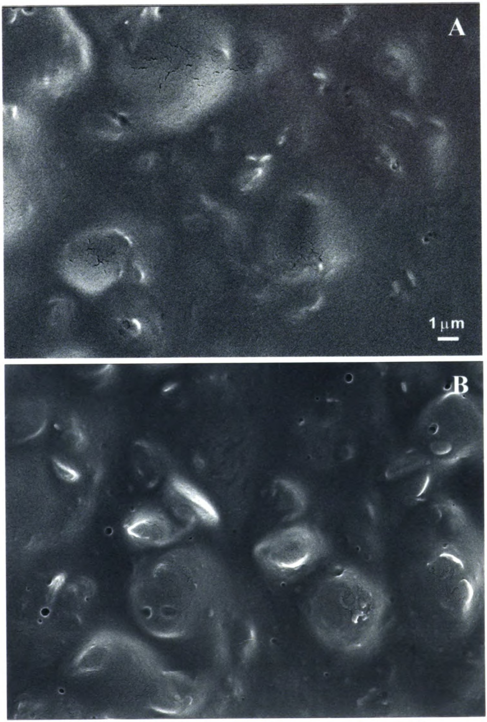


Figure 8. SEM of the Top Surface of a Lactic Acid Casein (50%)-Sorbitol (45%)-Candelilla Wax (5%)(top) and a Lactic Acid Casein (50%)-Sorbitol (35%)-Candelilla Wax (15%)(bottom) Edible Film

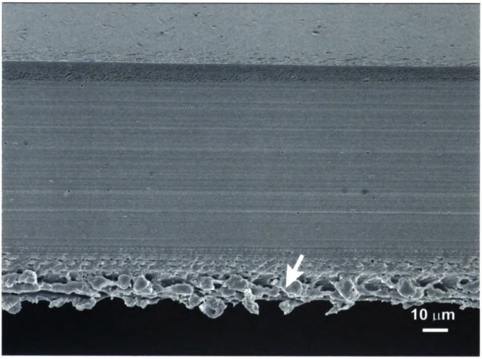


Figure 9. SEM of the Cross Sectional Surface of a Lactic Acid Casein (50%)-Sorbitol (50%) Edible Film (arrow represents perceived sorbitol layer)

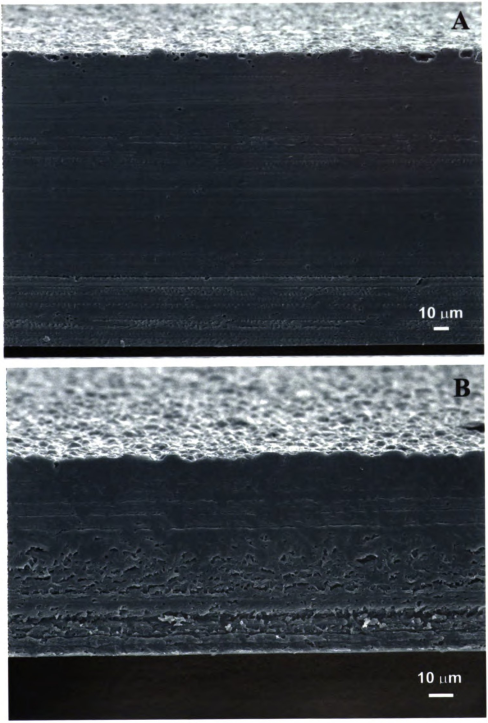


Figure 10. SEM of the Cross Sectional Surface of a Lactic Acid Casein (50%)-Sorbitol (45%)-Carnauba Wax (5%)(top) and a Lactic Acid Casein (50%)-Sorbitol (35%)-Carnauba Wax (15%)(bottom) Edible Film

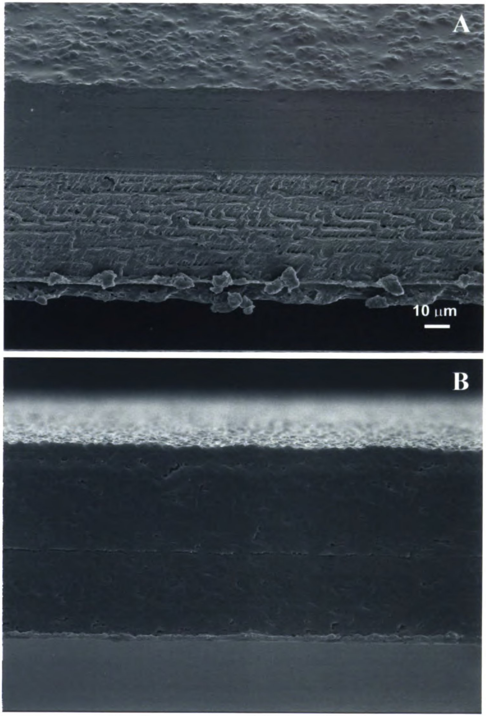


Figure 11. SEM of the Cross Sectional Surface of a Lactic Acid Casein (50%)-Sorbitol (45%)-Candelilla Wax (5%)(top) and a Lactic Acid Casein (50%)-Sorbitol (35%)-Candelilla Wax (15%)(bottom) Edible Film

layer does not show up in films containing wax, possibly due to the wax content at the top surface, not allowing this cracking to occur.

In cross sections of films containing wax, less of a layering structure was evident in films containing 5% wax as compared to films with 15% wax. A definite change in appearance occurs near the midpoint of the cross section, in films containing 15% wax, that is not as apparent in films containing 5% wax. One possible explanation could be the result of the higher wax content altering the drying of the film, producing this effect. Another possibility is that 15% wax is a high enough content to show a separation of phases beginning to occur.

3.3 Thermal Characteristics

DSC results of the lactic acid casein powder showed a rather broad endothermic peak, with it's melt occurring between 100 and 110°C (Figure 12, Table 9). The change in heat flow was relatively low, with a maximum around -0.82 W/g. Sorbitol possessed a narrow endothermic peak around 100°C (Figure 12, Table 9). However, it's change in heat flow was higher, around -4.5 W/g. DSC analysis of carnauba wax and candelilla wax where similar to that of Donhowe and Fennema (1993), with carnauba wax and candelilla wax having fairly narrow endothermic peaks between 82 and 86°C and 68 and 73°C respectively (Figure 12, Table 9). The change in heat flow for the waxes were around -3.1 and -2.4 W/g for carnauba and candelilla wax respectively.

Thermal analysis of lactic acid casein (50%)-sorbitol (50%) edible films showed one broad endothermic peak in the temperature range of 100 to 110°C and a change in heat flow of -0.61 W/g (Figure 13, Table 9), corresponding more closely to that of

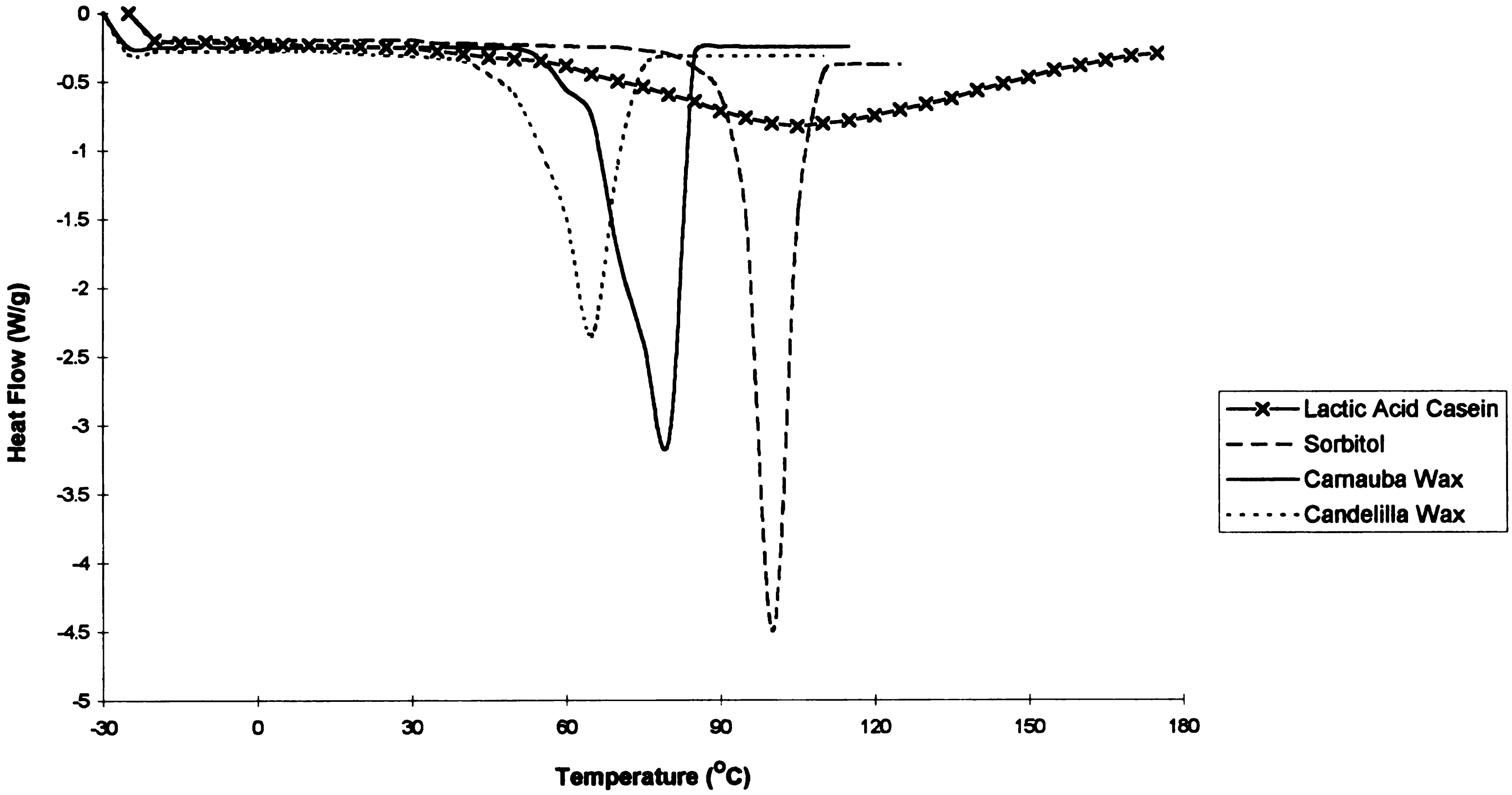


Figure 12. Differential Scanning Calorimetry of Primary Film Forming Components

Table 9. Differential Scanning Calorimetry Results of Primary Film Forming Components and Casein-Based Edible Films

	Approximate Melt Range (°C)	**Approximate Change in Heat Flow (W/g)**
Film Components		
Lactic Acid Casein	100-110	-0.82
Sorbitol	98-102	-4.5
Carnauba Wax	82-86	-3.1
Candelilla Wax	65-73	-2.4
Films		
Lactic Acid Casein (50%)-Sorbitol (50%)	100-110	-0.61
Lactic Acid Casein (50%)-Sorbitol (35%)-Carnauba Wax (15%)	85, 105-110	-0.8, -0.55
Lactic Acid Casein (50%)-Sorbitol (35%)-Candelilla Wax (15%)	65, 110-115	-0.7, -0.55

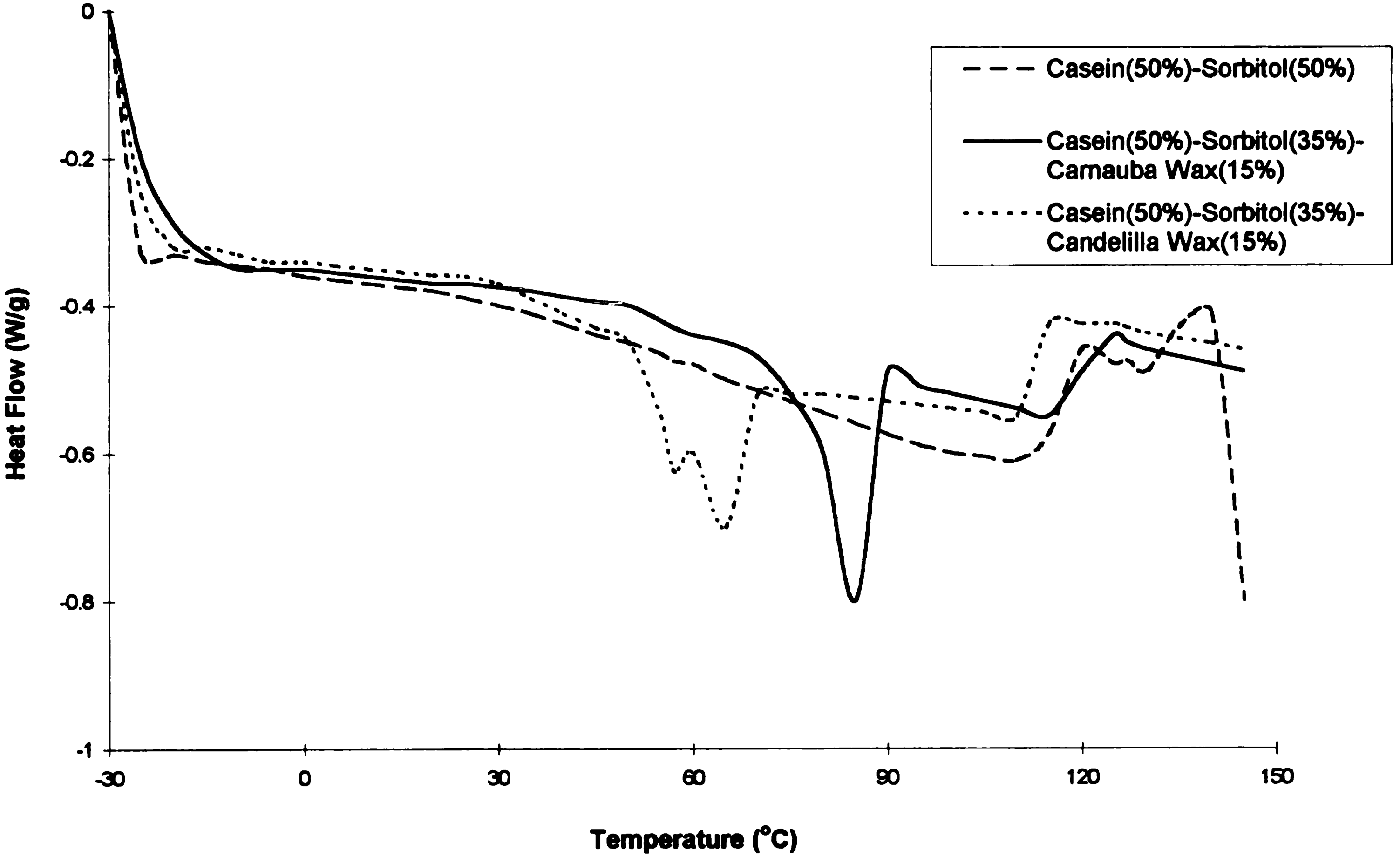


Figure 13. Differential Scanning Calorimetry of Casein-Based Edible Films

lactic acid casein powder than sorbitol. However, film samples containing wax showed what appears to be two endothermic peaks. Lactic acid casein (50%)-sorbitol (35%)-carnauba wax (15%) films showed one definite narrow endothermic peak in the temperature range similar to that of carnauba wax, and one possible broad peak in the range similar to the lactic acid casein-sorbitol films (Figure 13, Table 9). The change in heat flow in both peaks are similar to that of lactic acid casein, being -.08 and -0.55 W/g for the first and second peak respectively. The same is shown for lactic acid casein (50%)-sorbitol (35%)-candelilla wax (15%) films, where two endothermic peaks appear (Figure 13, Table 9), one similar to that of candelilla wax and one similar to the lactic acid casein-sorbitol film. Again, with the change in heat flow for both being similar to lactic acid casein, -0.7 and -0.55 for the first and second peak respectively. These results coincide closely with those from the SEM studies, where a partial separation of the two phases may have occurred. Partial in these DSC studies because we do not see a change in heat flow of the films containing wax to be similar to the waxes on their own.

3.4 Moisture Sorption Isotherm

The GAB model curve corresponded closely with experimental data for the lactic acid casein (50%)-sorbitol (50%) films (Figure 14, Table 10). The GAB model constants for the lactic acid casein film were 4.778, 22.739, and 1.034 for W_m, C, and k respectively. From these results we see a slow increase in the equilibrium moisture content of the lactic acid casein-sorbitol films from 0 to about 70% RH, followed by exponential growth peaking at around 85% RH. From this you can predict an increase in permeability and elongation properties, and a decrease in tensile strength will occur in these films near the

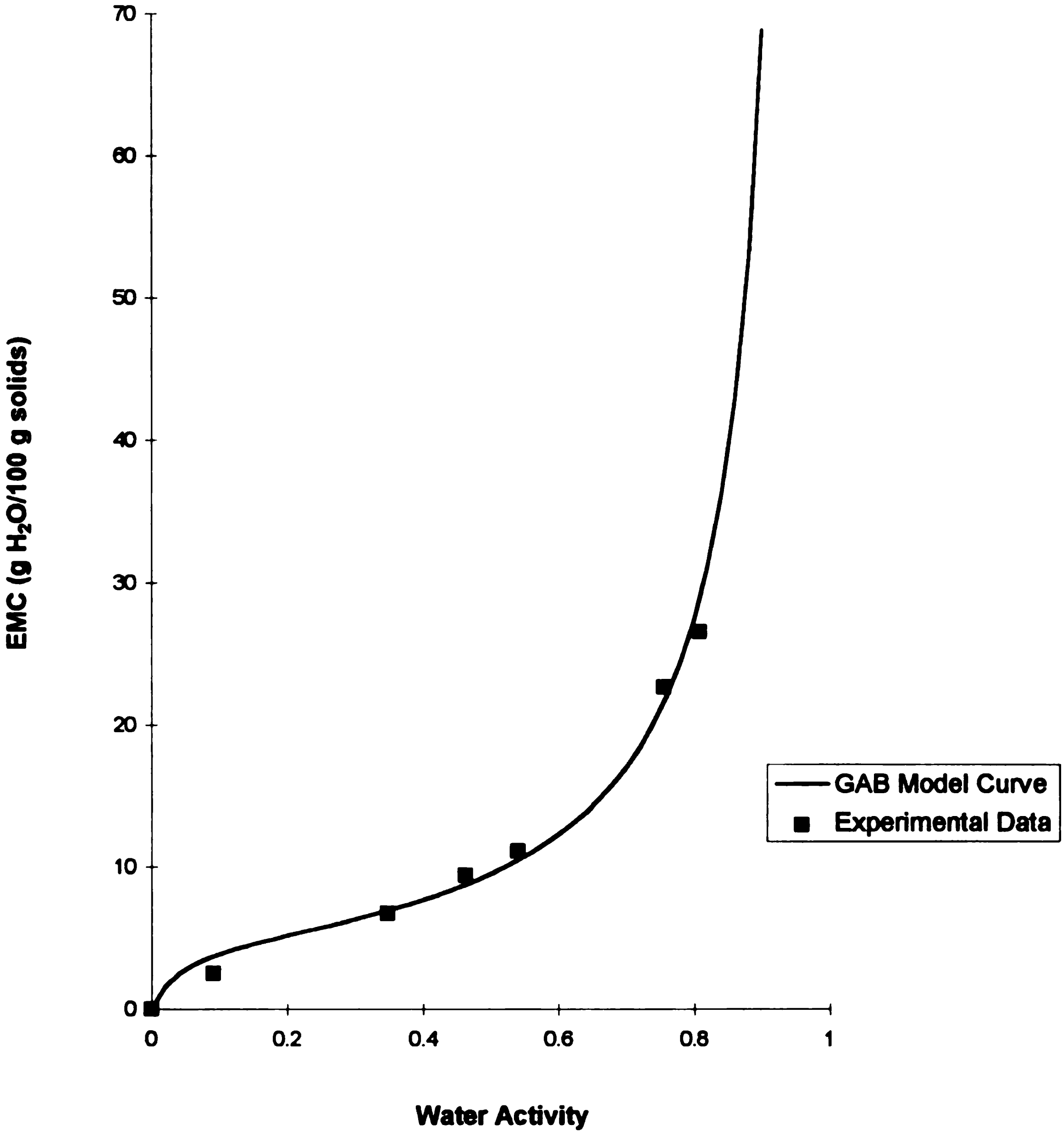


Figure 14. Moisture Sorption Isotherm of Lactic Acid Casein (50%)-Sorbitol (50%) Edible Films (Tested at 23°C)

Table 10. Experimental Data for Moisture Sorption Isotherm

	Lactic acid casein powder (50%)-sorbitol (50%)	**Rennet casein powder (58%)-glycerol (42%)**
RH (%)	**EMC (g H_2O/100g solid)**	**EMC (g H_2O/100g solid)**
0	0	0
9.1	2.5	16.4
34.8	6.7	25.9
46.3	9.4	27.4
54.0	11.1	28.3
75.5	22.6	39.2
80.8	26.5	45.9

70% RH point. The added moisture present in these films above this level would act to further plasticize the films.

3.5 Water Vapor Permeability

3.5.1 Wax Content

It was found that as the content of carnauba wax was increased from 5 to 15% in lactic acid casein (50%)-sorbitol-wax edible films (sorbitol and wax comprise the other 50%) there was a significant decrease ($p < 0.05$) in WVP at both 50 and 70% RH (Figure 15, Table 11). This can be explained by the increased surface area of the film covered by the carnauba wax, with less of the casein exposed, as seen in the SEM's (Figure 7). This same was also seen with the increase in candelilla wax content (Figure 16, Table 11). At 50% RH, no significant difference ($p < 0.05$) in WVP was observed between the lactic acid casein (50%)-sorbitol (50%) films and those containing up to 10% carnauba wax (Figure 15, Table 11). However, WVP was better in films containing 15% carnauba wax. It was expected that WVP would decrease as carnauba wax content increased because of the good water barrier properties carnauba wax possesses, however, this did not occur. This is most likely a result of the difficulty in the incorporation of carnauba wax into the films, due to it's high melting point (Section 3.1). Due to this, more disruption in the film matrix occurred, creating an increased number of void spaces at the protein-wax interface. This is further seen at 70% RH, a higher moisture driving force, where the WVP was significantly higher ($p < 0.05$) in films containing carnauba wax compared to films with no wax.

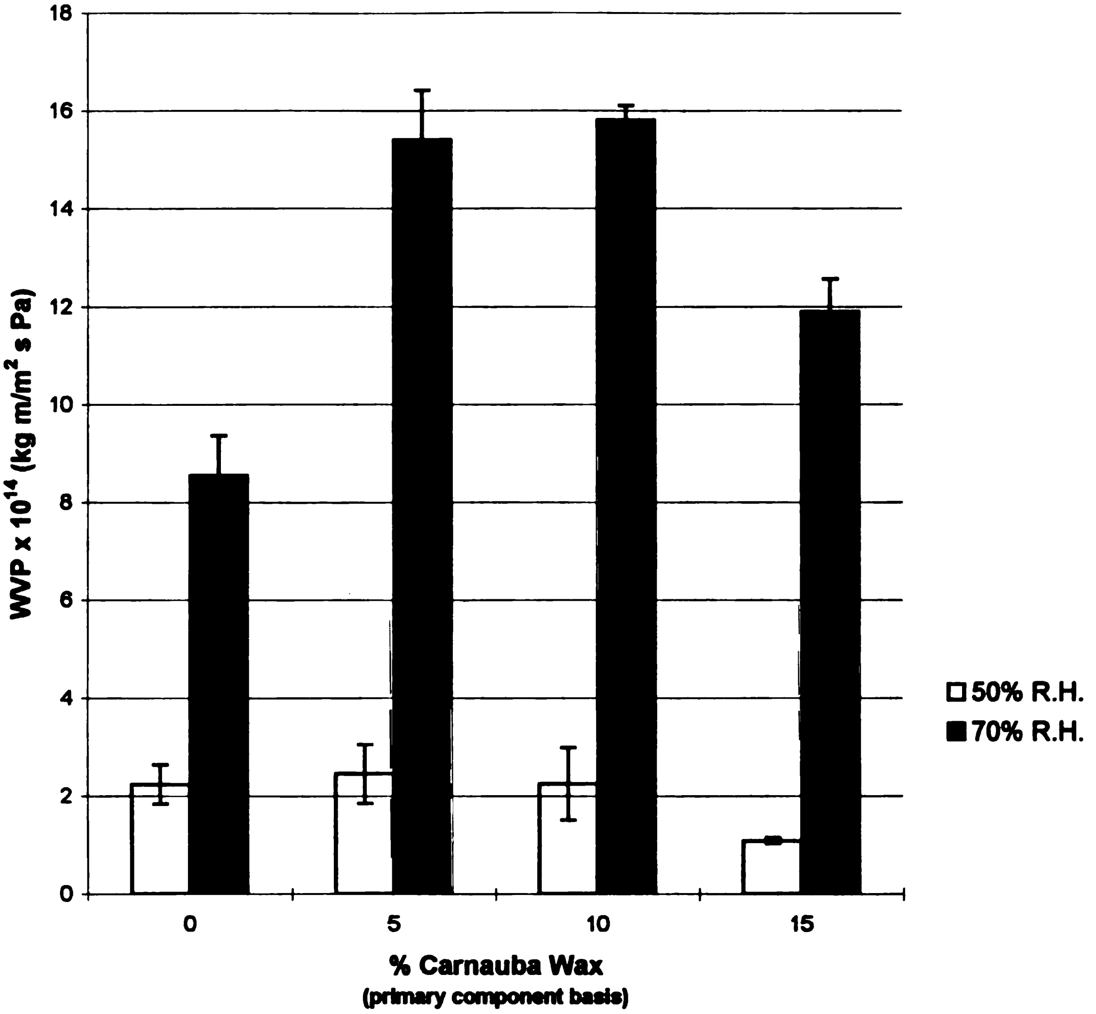


Figure 15. Water Vapor Permeability (WVP) of Lactic Acid Casein (50%)-Sorbitol-Carnauba Wax Edible Films
(Tested at 37.8°C, thickness = 0.1mm)

Table 11. Water Vapor Permeability[1] of Lactic Acid Casein (50%)-Sorbitol-Wax Edible Films[2]

Wax Type(%)	**WVP x 10^{14} (kg m/m^2 s Pa) @ 50% RH**	**WVP x 10^{14} (kg m/m^2 s Pa) @ 70% RH**
No Wax (0)	2.23 ± 0.60^{ab}	8.54 ± 0.82^{a}
Carnauba (5)	2.45 ± 0.60^{a}	17.91 ± 1.02^{b}
(10)	2.26 ± 0.74^{ab}	18.32 ± 0.30^{b}
(15)	1.08 ± 0.06^{c}	13.76 ± 0.66^{c}
Candelilla (5)	1.42 ± 0.26^{bd}	6.78 ± 0.61^{d}
(10)	1.01 ± 0.22^{cd}	6.00 ± 1.32^{de}
(15)	0.68 ± 0.16^{cd}	5.14 ± 0.88^{e}

[a-e] Different letter columnwise denotes significant difference ($p < 0.05$)

[1] All WVP tests done at 37.8°C

[2] Sorbitol and Wax comprised the remaining 50% of the films (on a dry basis), thickness of films were 0.104 ± 0.007 mm (4.09 ± 0.03 mils)

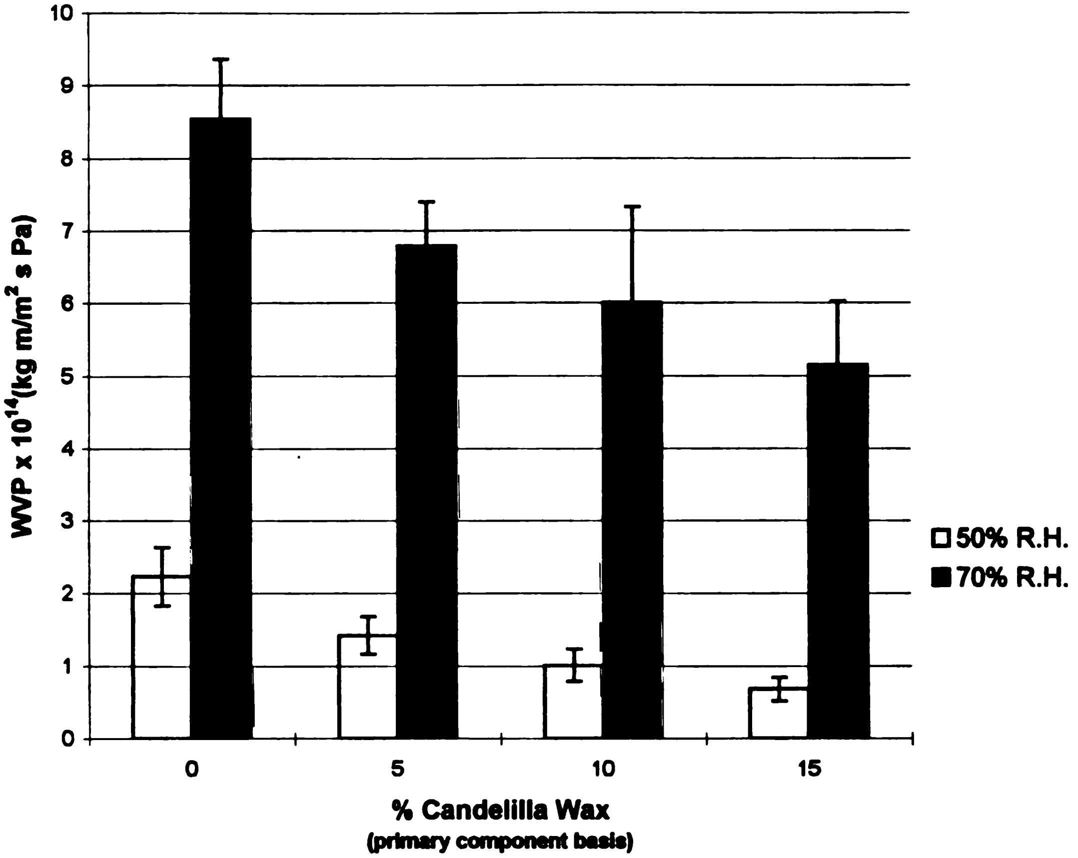


Figure 16. Water Vapor Permeability (WVP) of Lactic Acid Casein(50%)-Sorbitol-Candelilla Wax Edible Films (Tested at 37.8°C, thickness = 0.1mm)

At both 50 and 70% RH, with the addition of candelilla wax to films a significant decrease ($p < 0.05$) in WVP occurred versus those without wax, (Figure 16, Table 11). This further supports carnauba wax being more difficult to incorporate into films, causing disruptions in the film matrix.

These results show that these films are more influenced by the properties of lactic acid casein. This is most likely due to the relatively low concentration of wax able to be incorporated into the films. With the low wax content and the high percentage of the lactic acid casein, with it's hydrophilic characteristics, these films are able to adsorb moisture readily. Another factor in the high WVP is the large concentration of plasticizer required in these films, breaking up some of the intermolecular bonding, allowing for the easier diffusion of water.

These results help to support SEM and DSC findings where a partial, not complete, phase separation of the wax occurred. If a complete separation had occurred the water barrier properties should have been closer to that of wax films, because of a solid wax layer. However, these show WVP properties more similar to those of the lactic acid casein-sorbitol films, meaning many of the polar reactive groups present in lactic acid casein (peptide, amido, and carboxyl groups) are found at the top surface of the films commingled with wax. WVP of these films still are much higher than films considered to be good water barriers like HDPE, carnauba wax, and candelilla wax (Table 12). When comparing WVP of different films the RH and temperature of testing must be taken into consideration.

Table 12. Water Vapor Permeability (WVP) Comparison of Lactic Acid Casein (50%)-Sorbitol-Wax Edible Films to Other Films (Edible and Synthetic)

Film Type[1]	WVP x 10^{14} (kg m/m^2 s Pa)	Test Conditions	Reference
LAC-S(45)-CR(5)	2.45	37.8°C, 50% RH	Present
LAC-S(45)-CR(5)	17.91	37.8°C, 70% RH	Present
LAC-S(35)-CR(15)	1.08	37.8°C, 50% RH	Present
LAC-S(35)-CR(15)	13.76	37.8°C, 70% RH	Present
LAC-S(45)-CN(5)	1.42	37.8°C, 50% RH	Present
LAC-S(45)-CN(5)	6.78	37.8°C, 70% RH	Present
LAC-S(35)-CN(15)	0.68	37.8°C, 50% RH	Present
LAC-S(35)-CN(15)	5.14	37.8°C, 70% RH	Present
LAC-S(50)	2.23	37.8°C, 50% RH	Present
LAC-S(50)	8.54	37.8°C, 70% RH	Present
LAC-S(50)	52.07	37.8°C, 90% RH	Chick (1996)
Carnauba Wax	0.033	25°C, 100% RH	Donhowe and Fennema (1993)
Candelilla Wax	0.018	25°C, 100% RH	Donhowe and Fennema (1993)
HDPE	0.02	37.8°C, 90% RH	Smith (1986)
Nylon 6	8.21	37.8°C, 90% RH	Tubrity & Foster (1986)

[1] LAC - Lactic Acid Casein, S - Sorbitol, CR - Carnauba Wax, CN - Candelilla Wax, HDPE - High Density Polyethylene. Sorbitol and Wax comprised remaining 50% of film (dry basis), number in parenthesis is that percentage.

3.5.2 Wax Type

At 50% RH, candelilla wax incorporated into lactic acid casein films provided a significantly better ($p < 0.05$) water barrier than films made with carnauba wax at 5 and 10% wax, however, no significant difference was observed at 15% wax (Figure 17, Table 11). At 70% RH, the WVP of films containing candelilla wax were significantly lower ($p < 0.05$) than films containing carnauba wax, at all wax levels (Figure 17, Table 11). This can be explained by the higher hydrocarbon content in candelilla wax (about 50% in candelilla wax and 1.5 to 3% in carnauba wax) that form on the surface, while carnauba wax is predominantly made up of wax esters (about 85% compared to about 29% in candelilla wax), which contain a polar group. Also, the difficulty of incorporating carnauba wax into the lactic acid casein films probably plays a more significant role, increasing the void spaces. WVP was close to two times lower in films containing candelilla wax at 50% RH compared to films with carnauba wax, while it was almost three times lower at 70% RH. Which again, could be due to increased sorption of water by the ester groups in carnauba wax and a more efficient barrier created by the hydrocarbon chains in candelilla wax, but most likely by the increased void spaces in the films containing carnauba wax. These are consistent with findings by Donhowe and Fennema (1993) in wax films and Shellhammer and Krochta (1997) in emulsion films when comparing carnauba to candelilla wax.

3.5.3 RH Effect

With an increase in RH from 50 to 70%, the WVP In lactic acid casein (50%)-sorbitol (50%) films increased 3.8 times, from 2.23 to 8.54 kg m/m^2 s Pa. Chick (1996)

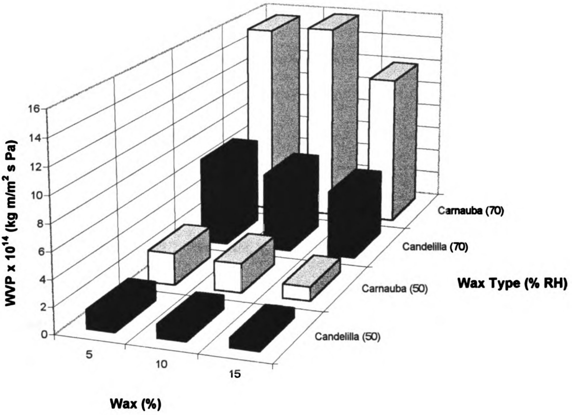


Figure 17. Water Vapor Permeability (WVP) Comparison of Lactic Acid Casein (50%)-Sorbitol-Carnauba Wax and Lactic Acid Casein (50%)-Sorbitol-Candelilla Wax Edible Films
(Tested at 37.8°C, thickness = 0.1mm)

found the WVP in lactic acid casein (50%)-sorbitol (50%) edible films (thickness 0.203 mm) to be 52.07 kg m/m^2 s Pa, tested at 23°C and 90% RH. By adding this result to the findings from this study we observe an exponential rise in WVP of these films (Figure 18). These results correspond closely to the isotherm model of these films.

As RH was increased from 50 to 70%, the WVP of lactic acid casein (50%)-sorbitol-carnauba wax edible films increased 7.3, 8.1, and 12.7 times for films containing 5, 10, and 15% carnauba wax respectively. In films containing candelilla wax WVP increased 4.8, 5.9, and 7.6 times for 5, 10, and 15% candelilla wax respectively. This shows that the increasing RH had more of an effect on WVP in films containing carnauba wax. Again, because of the incorporation problems. Increasing RH having more of an effect on films as the wax content increased. This is probably the result of increased disruptions in the film matrix, due to the presence of wax.

3.6 Oxygen Permeability

3.6.1 Wax Content

no statistical difference ($p < 0.05$) was witnessed in OP with the addition of wax, either candelilla or carnauba wax, to lactic acid casein-sorbitol films (Table 13). This is similar to results found by Gennadios *et al.* (1993) with WG-AM edible films. Kim and Ustunol (1997) witnessed a decrease in OP with the addition of candelilla wax in WPI and WPC-based edible films. As wax content increased from 5 to 15%, either carnauba or candelilla wax, no significant difference ($p < 0.05$) in OP was witnessed. This is similar to results found by Kim and Ustunol (1997) with WPI and WPC-candelilla wax edible films.

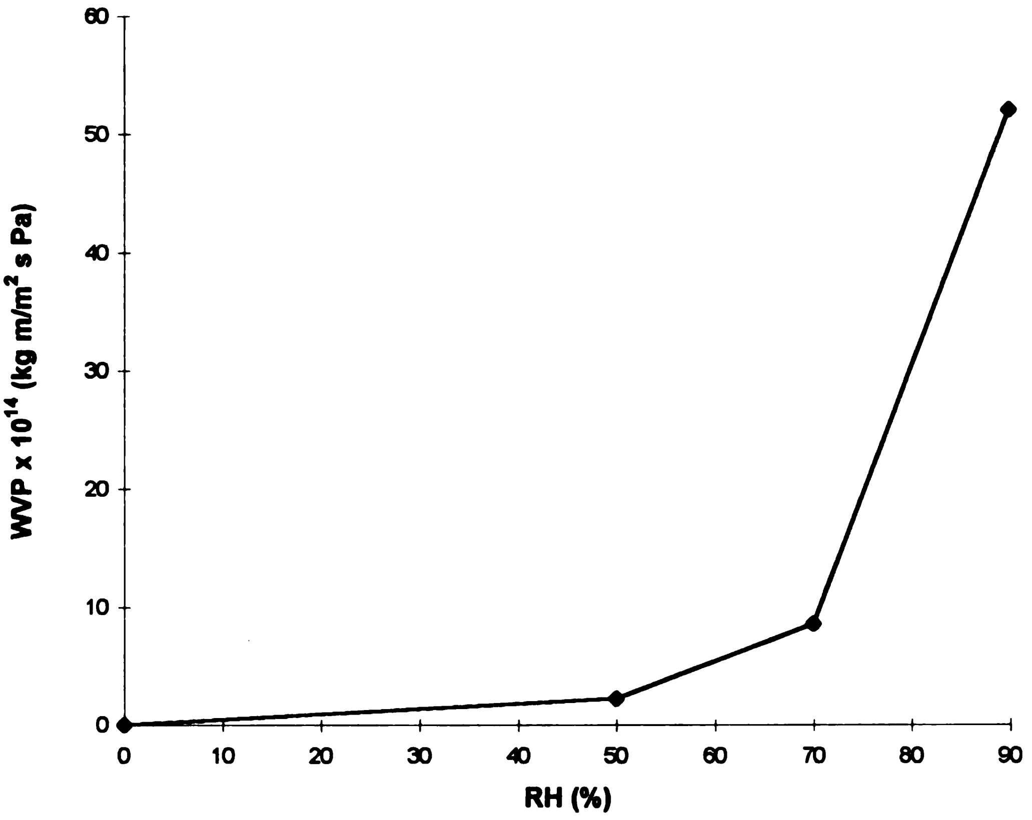


Figure 18. Water Vapor Permeability (WVP) of Lactic Acid Casein (50%)-Sorbitol (50%) Edible Films as Effected by Relative Humidity (RH)
(Tested at 37.8°C, thickness = 0.1mm)

Table 13. Oxygen Permeability (OP)[1] of Lactic Acid Casein (50%)-Sorbitol-Wax Edible Films[2]

	OP x 10^{20}(m^3 m/m^2 s Pa)	
Wax Content (%)	**Carnauba Wax**	**Candelilla Wax**
0	0.48 ± 0.16^a	0.48 ± 0.16^a
5	0.31 ± 0.09^a	0.35 ± 0.07^a
10	0.38 ± 0.12^a	0.44 ± 0.04^a
15	0.63 ± 0.24^a	0.71 ± 0.31^a

[a] Different letter throughout table denotes significant difference ($p < 0.05$)

[1] All OP tests done at 23°C

[2] Sorbitol and Wax comprised the remaining 50% of the films (on a dry basis), thickness of films were 0.111 ± 0.009 mm (4.37 ± 0.04 mils)

It was expected that OP would increase with the addition of wax , because of their poor oxygen barrier properties. Although, there may be more void areas near the surface of films containing wax, where the wax is present in larger concentrations (from the SEM studies), a predominantly protein, defect free matrix could seen throughout the rest of the films' thickness. These results coincide with results from SEM studies, which show that the bottom portion of these films probably consist of a lactic acid casein-sorbitol layer, creating the good oxygen barrier. While the wax is found predominantly near the top portion of the film in perhaps a partially separated emulsion form. Since, OP is mostly dependent on diffusion, because oxygen is less compressible then water (Banker, 1966), these results also support the presence of a good matrix system.

The OP properties of these films fall in between those possessed by EVOH, considered an excellent oxygen barrier, and nylon 6, a very good oxygen barrier. While they proved to be much better barriers than carnauba and candelilla wax on their own (Table 14).

3.6.2 Wax Type

Wax type, carnauba or candelilla wax, did not effect the oxygen barrier properties of lactic acid casein-sorbitol-wax edible films. Since the lactic acid casein matrix found near the bottom surface is thought to have been the predominant influence on OP, the wax did not play a significant role in the oxygen barrier properties. Even if they did play a role in the OP, differences between the waxes would not be expected because of the findings of Donhowe and Fennema (1993), showing no significant differences between carnauba wax and candelilla wax films.

Table 14. Oxygen Permeability (OP) Comparison of Lactic Acid Casein (50%)-Sorbitol-Wax Films to other Films (Edible and Synthetic)

Film Type[1]	**O x 10^{-20} (m^3 m/m^2 s Pa)**	**Test Conditions**	**Reference**
LAC(50)-S(50)	0.475	23°C, 0% RH	Present
LAC-S(35)-CR(15)	0.625	23°C, 0% RH	Present
LAC-S(35)-CN(15)	0.706	23°C, 0% RH	Present
Carnauba Wax	180	23°C, 0% RH	Donhowe and Fennema (1993)
Candelilla Wax	200	23°C, 0% RH	Donhowe and Fennema (1993)
HDPE	494	23°C, 50% RH	Smith (1986)
EVOH	0.076	23°C, 0% RH	Foster (1986)
Nylon 6	11.7	23°C, 0% RH	Tubrity & Sibilia (1986)

[1] LAC - Lactic Acid Casein, S - Sorbitol, CR - Carnauba Wax, CN - Candelilla Wax, HDPE - High Density Polyethylene. Sorbitol and Wax comprised remaining 50% of film (dry basis), number in parenthesis is that percentage.

3.6.3 RH Effect

As RH increase from 0 to 50%, no significant difference ($p < 0.05$) in OP was observed in any of the edible film samples, with or without wax. At 70% RH, however, a significant increase ($p < 0.05$) in OP occurred in all samples (Figure 19, Table 15). In general, a minimum OP occurred at 35% RH for the film samples. With PET there was a decreasing trend, with a minimum at 50% RH, followed by an increase at 70% RH. However, there was no significant difference ($p < 0.05$) in OP as RH increased from 0 to 70% with the PET.

Lactic acid casein-sorbitol and lactic acid casein-sorbitol-wax edible films acted like nylon 6 (Gavara and Hernandez, 1994; Hernandez, 1994), where solubility is decreasing until around the 35% RH region. Then, around the 70% RH region the matrix is probably being disrupted by clustering water allowing for the greater OP. This also coincides with the moisture sorption isotherm results, where a large increase in moisture content occurred around 70% RH. This same result was witnessed in PET films tested, with a minimum around 50%. However, the increase was only slight following this point and more studies at higher RH's should be done to conclude this increasing phenomenon.

3.7 Mechanical Properties

3.7.1 Tensile Strength

At a 50% RH, the addition of 5 to 15% wax, either carnauba or candelilla, was found to have no significant effect ($p < 0.05$) on the TS of lactic acid casein (50%)-sorbitol-wax films compared to films without wax (Figures 20 and 21, Table 16). At 75% RH, with the addition of carnauba wax, a significant increase in TS was observed at 10

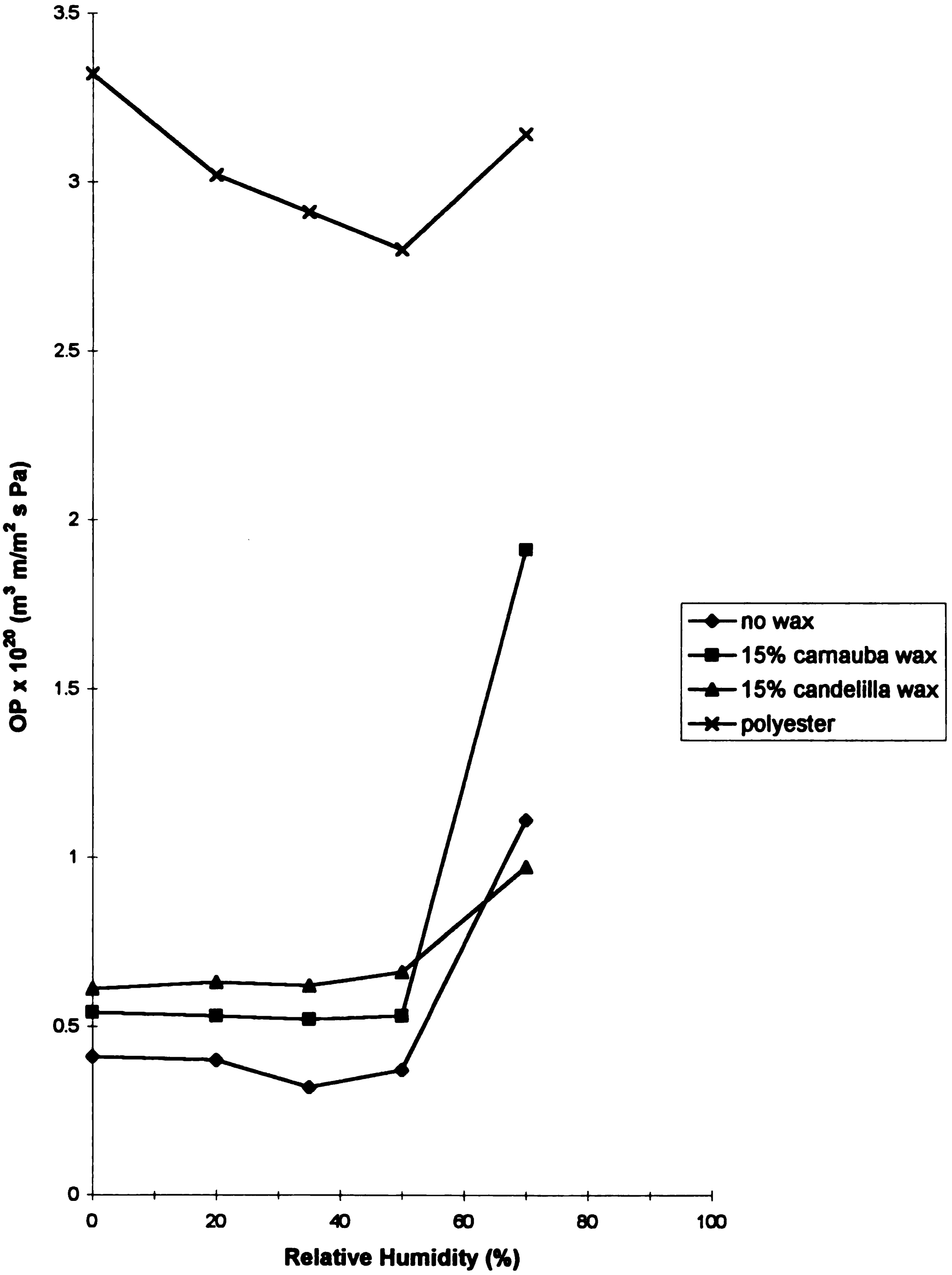


Figure 19. Oxygen Permeability (OP) of Lactic Acid Casein (50%)-Sorbitol-Wax Edible Films

(Tested at 23°C, sorbitol and wax comprise 50% of film)

Table 15. Oxygen Permeability (OP)[1] of Lactic Acid Casein (50%)-Sorbitol (35%)-Wax (15%) Edible Films[2] (dry basis) as Effected by Relative Humidity (RH)

	OP x 10^{20}(m^3 m/m^2 s Pa)			
RH (%)	**No Wax**	**Carnauba Wax**	**Candelilla Wax**	**Polyester**
0	0.48 ± 0.16^{a}	0.63 ± 0.24^{a}	0.71 ± 0.31^{ab}	3.84 ± 0.20^{a}
20	0.46 ± 0.13^{a}	0.61 ± 0.23^{a}	0.73 ± 0.01^{a}	3.50 ± 0.10^{a}
35	0.37 ± 0.09^{a}	0.60 ± 0.23^{a}	0.72 ± 0.06^{a}	3.37 ± 0.09^{a}
50	0.43 ± 0.07^{a}	0.63 ± 0.28^{a}	0.76 ± 0.07^{a}	3.24 ± 0.08^{a}
70	1.29 ± 0.29^{b}	2.21 ± 0.60^{b}	1.12 ± 0.26^{b}	3.63 ± 0.09^{a}

[a,b] Different letter columnwise denotes significant difference ($p < 0.05$)

[1] All OP tests done at 23°C

[2] Thickness of films were 0.111 ± 0.009 mm (4.37 ± 0.04 mils)

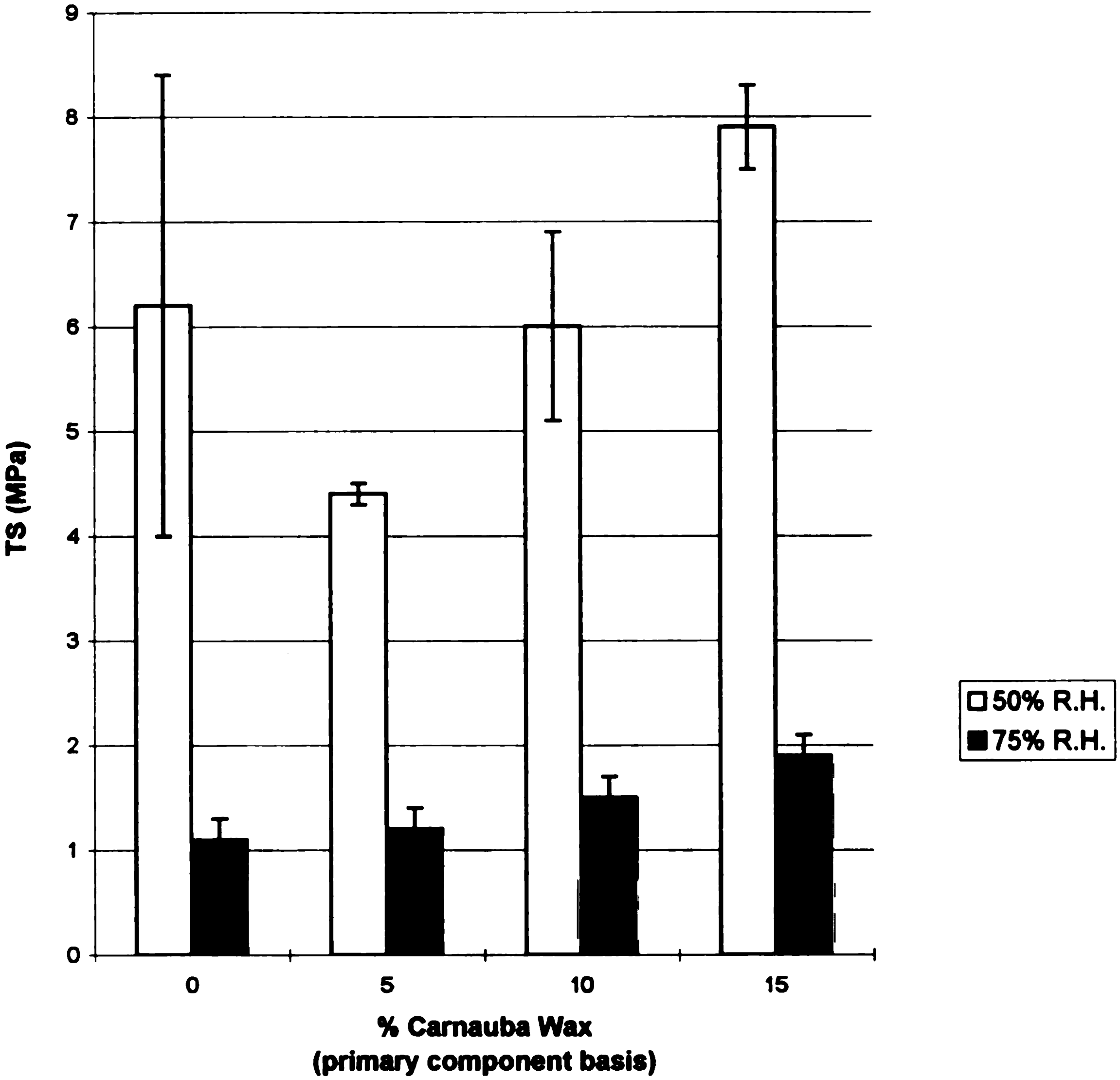


Figure 20. Tensile Strength (TS) of Lactic Acid Casein (50%)-Sorbitol-Carnauba Wax Edible Films

(Tested at 23°C)

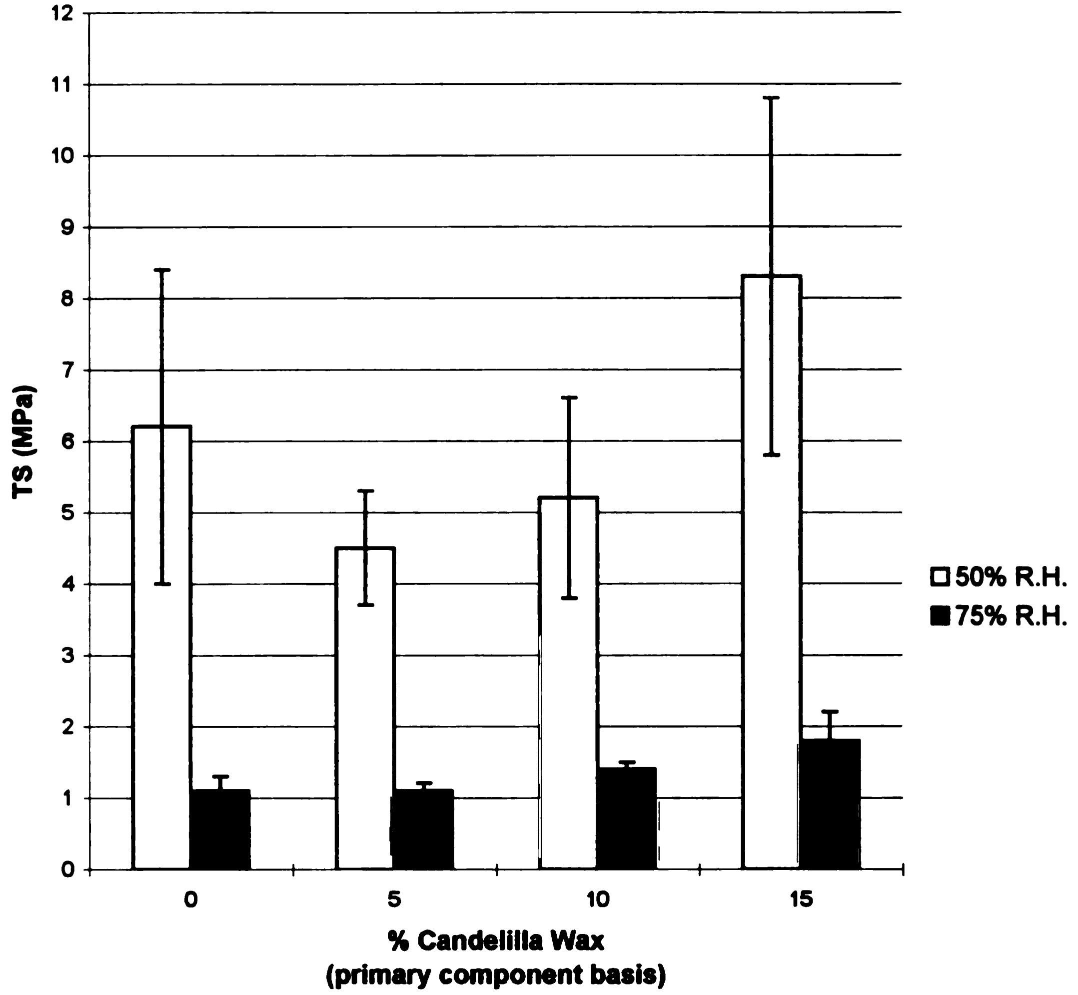


Figure 21. Tensile Strength (TS) of Lactic Acid Casein (50%)-Sorbitol-Candelilla Wax Edible Films
(Tested at 23°C)

Table 16. Tensile Strength (TS)[1] of Lactic Acid Casein (50%)-Sorbitol-Wax Edible Films[2]

Wax Type(%)	TS (MPa) @ 50% RH	TS (MPa) @ 75% RH
No Wax (0)	6.2 ± 2.2^{abc}	1.1 ± 0.2^{a}
Carnauba (5)	4.4 ± 0.1^{a}	1.2 ± 0.2^{ab}
(10)	6.0 ± 0.9^{b}	1.5 ± 0.2^{b}
(15)	7.9 ± 0.4^{c}	1.9 ± 0.2^{c}
No Wax (0)	6.2 ± 2.2^{a}	1.1 ± 0.2^{a}
Candelilla (5)	4.5 ± 0.8^{a}	1.1 ± 0.1^{a}
(10)	5.2 ± 1.4^{a}	1.4 ± 0.1^{ab}
(15)	8.3 ± 2.5^{a}	1.8 ± 0.4^{b}

[a,b] Different letters in columnwise wax groups denote significant difference ($p < 0.05$)

[1] All TS tests done at 23°C

[2] Sorbitol and Wax comprised the remaining 50% of the films (on a dry basis)

and 15% wax, compared to films without wax. A significant increase ($p < 0.05$) in TS was also observed in films containing candelilla wax at a 15% level, and a RH of 75%. Kim and Ustunol (1997) found and increase in TS with the addition of candelilla wax to WPI and WPC edible films, at 23°C and 50% RH.

A significant increase ($p < 0.05$) in TS occurred with an increasing concentration of wax at both RH's, except with candelilla wax at 50% RH (Figures 20 and 21, Table 16). However, an increasing trend was present in this case. In the lactic acid casein (50%)-sorbitol (50%) films TS is created by the protein matrix. The addition of wax at low levels caused a disruption in the protein matrix causing a decrease in TS, at 50% RH. As wax content increased a more uniform layer of wax was established, providing the films with an increased TS. At 70% RH, wax did not play as large a role in TS in these films due to the plasticization of the films by the increased moisture content. These results are contrary to those found by Shellhammer and Krochta (1997) who found a linear decrease in TS as either carnauba or candelilla wax content increased, in WPI-glycerol-wax emulsion films. Kim and Ustunol (1997), on the other hand, found no change in TS as candelilla wax content increased from 2 to 8%, in WPI and WPC-candelilla wax emulsion films. Differences in these results could be due to differences in the way the protein and waxes interact in emulsion or to differences in the protein. These results correspond well to those showing the possibility of a partial phase separation of the emulsion as wax content increases.

There were no significant differences ($p < 0.05$) in TS values between lactic acid casein (50%)-sorbitol-carnauba wax edible films and lactic acid casein (50%)-sorbitol-candelilla wax edible films at equivalent wax levels (Figure 22, Table 16). This is most

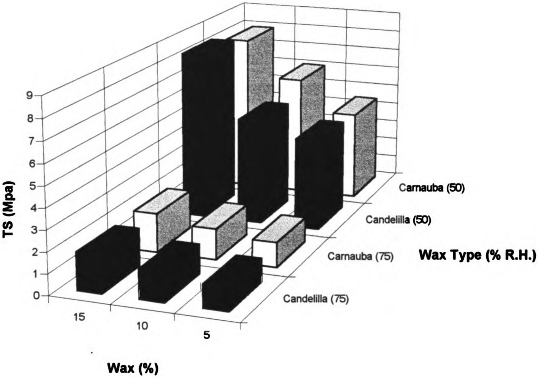


Figure 22. Tensile Strength (TS) Comparison of Lactic Acid Casein (50%)-Sorbitol-Carnauba Wax and Lactic Acid Casein (50%)-Sorbitol-Candelilla Wax Edible Films
(Tested at 23°C)

likely due to the relatively small concentration of wax within the films, 15% or less. Showing that differences between the waxes are not present at these relatively small concentrations, compared to protein. This is contrary to results found by Shellhammer and Krochta (1997), where WPI-glycerol-candelilla wax films were found to be weaker than WPI-glycerol-carnauba wax films. However, wax content in these films was much higher, ranging from 20 to 40%.

An increase in relative humidity, from 50 to 75%, produced a significant decrease ($p < 0.05$) in TS (Figure 22). This is again due to the increased moisture content in films at the higher RH, as seen by the moisture sorption isotherm results (Figure 14), further plasticizing the films. TS decreased around 4 times for films containing wax and 5.6 times for lactic acid casein (50%)-sorbitol (50%) edible films, with the increase in RH. This decrease shows the more hydrophobic properties that wax films possess, compared to lactic acid casein-sorbitol films alone.

3.7.2 Elongation

At 50% RH, films containing carnauba wax, at 10 and 15%, and candelilla wax, from 5 to 15%, significantly decreased ($p < 0.05$) the elongation of lactic acid casein (50%)-sorbitol films compared to films without wax. While at 75% RH, a significant decrease ($p < 0.05$) in elongation was witnessed with the addition of 10 and 15% of either wax (Table 17). This is most likely due to the wax disrupting the protein matrix, producing a less extensible film. At a 75% RH, the increase in moisture present in the films, further plasticizing the films, probably helps to counteract the addition of wax at the smaller concentrations. Similar results were reported by Shellhammer and Krochta (1997)

Table 17. Elongation[1] of Lactic Acid Casein (50%)-Sorbitol-Wax Edible Films[2]

Wax Type(%)	**Elongation(%) @ 50% RH**	**Elongation(%) @ 75% RH**
No Wax (0)	156 ± 6^{a}	167 ± 16^{a}
Carnauba (5)	159 ± 18^{a}	146 ± 20^{a}
(10)	55 ± 21^{b}	96 ± 4^{b}
(15)	31 ± 5^{b}	88 ± 9^{b}
No Wax (0)	156 ± 6^{a}	167 ± 16^{a}
Candelilla (5)	93 ± 30^{b}	156 ± 14^{a}
(10)	71 ± 26^{bc}	116 ± 17^{b}
(15)	37 ± 12^{c}	74 ± 21^{c}

[a-c] Different letters in columnwise wax gruops denote significant difference ($p < 0.05$)

[1] All Elongation tests done at 23°C

[2] Sorbitol and Wax comprised the remaining 50% of the films (on a dry basis)

and Kim and Ustunol (1997), where they found elongation to decrease with the addition of wax in whey protein films.

As wax content increased, carnauba or candelilla wax, at both 50 and 75% RH, a significant decrease in elongation was witnessed (Figures 23 and 24, Table 17). This again being due to the relative brittleness of wax and further disruption of the protein matrix. As has been seen, with the larger concentrations of wax a further separation of the wax is occurring. This would also cause the earlier film break, with the wax layer breaking, initiating the films failure. Kim and Ustunol (1997) found no change in elongation as wax content increased and WPC and WPI-candelilla wax films, however, the concentration of wax was relatively low, 2 to 8%.

No significant difference ($p < 0.05$) was observed in elongation between films containing carnauba wax and those containing candelilla wax, at equal wax levels and RH's, except at the 5% wax level and 50% RH (Figure 25). These results are most likely due to the waxes disrupting the protein matrix to similar degrees, and the relatively low wax concentration with the protein matrix giving the films their extension characteristics.

A significant increase ($p < 0.05$) in elongation was witnessed with an increase in RH from 50 to 70%, except those films without wax and with the addition of carnauba wax at 5% where no significant change occurred (Figure 25). An increase of around two times was witnessed in those films where a significant increase was noticed. This increase again being due to further plasticization of the films by the increased moisture at the higher RH.

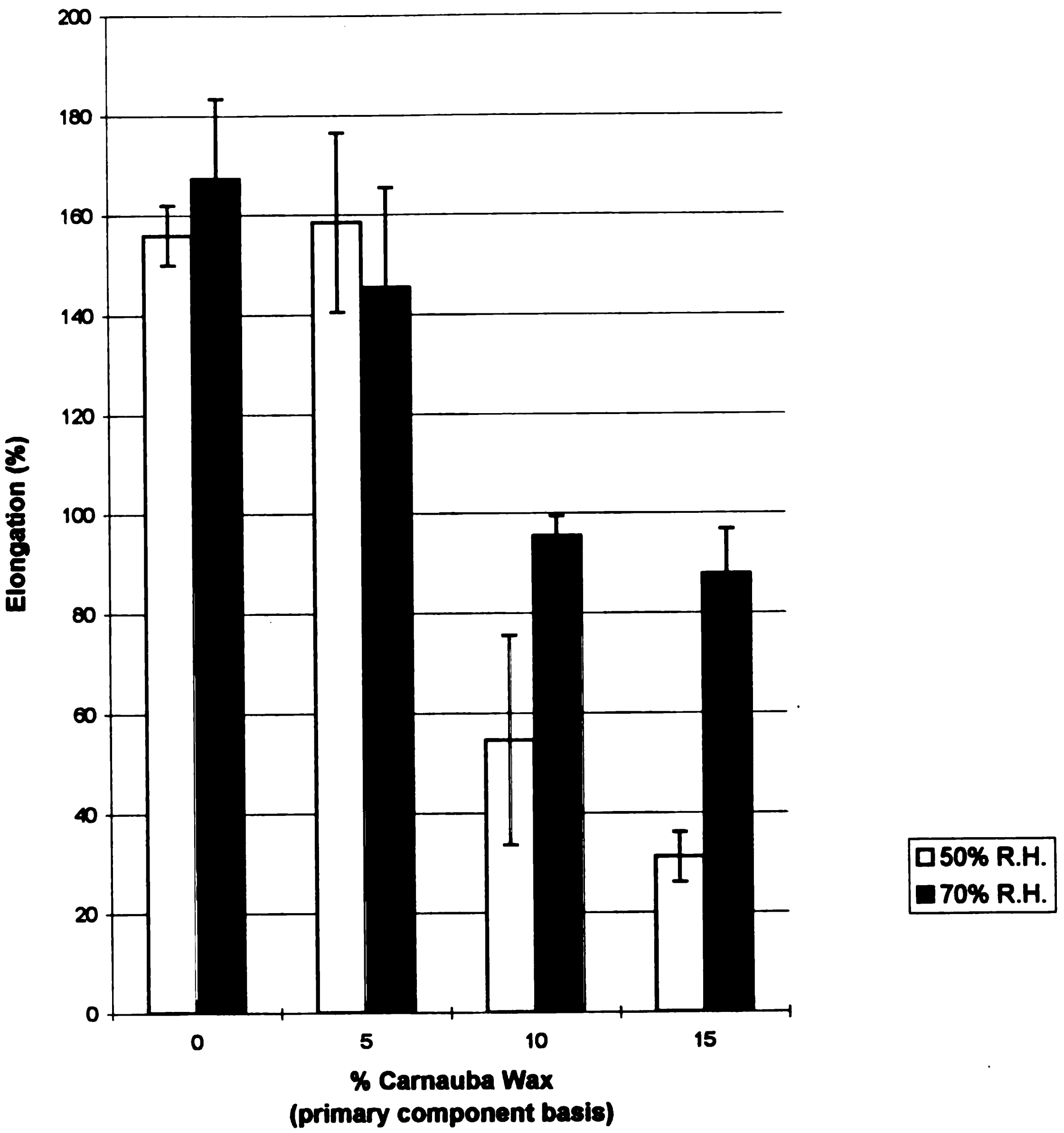


Figure 23. Elongation of Lactic Acid Casein (50%)-Sorbitol-Carnauba Wax Edible Films
(Tested at 23°C)

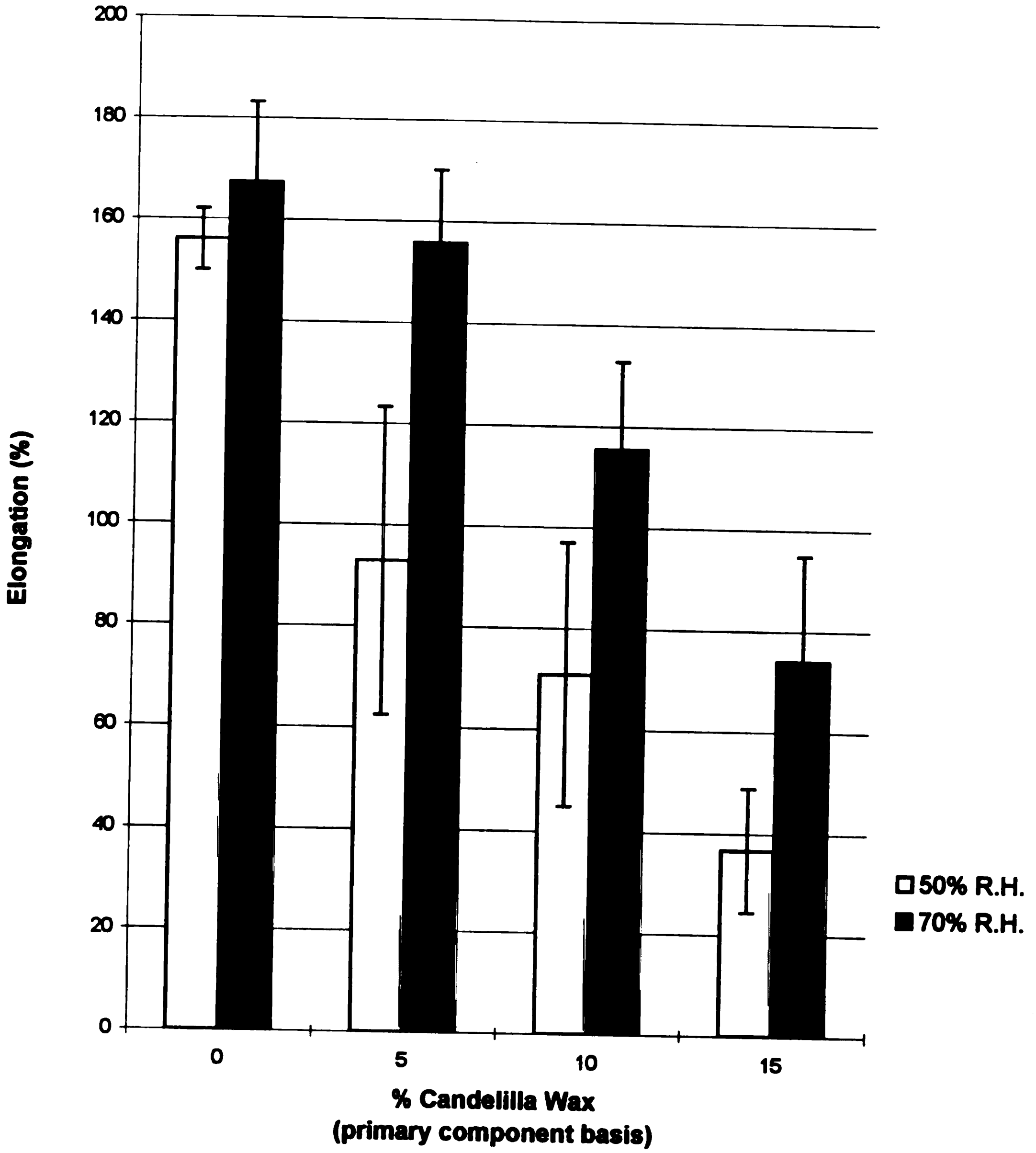


Figure 24. Elongation of Lactic Acid Casein(50%)-Sorbitol-Candelilla Wax Edible Films
(Tested at 23°C)

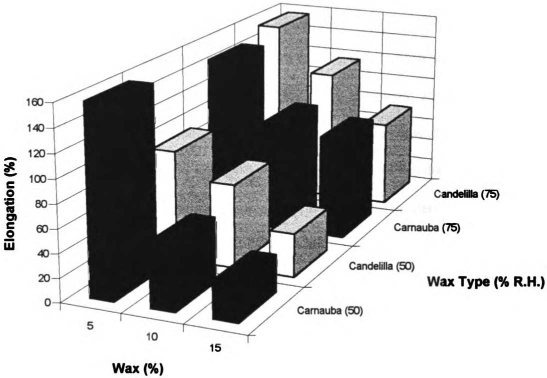


Figure 25. Elongation Comparison of Lactic Acid Casein (50%)-Sorbitol-Wax Edible Films
(Tested at 23°C)

3.7.3 Comparison to Other Polymers

As has been the case with edible films, they have generally been found to be much weaker than synthetic films (Table 18). Although the incorporation of wax did add some strength to these lactic acid casein-based films, the strongest are only comparable to the weaker of the LDPE films, and are much weaker than HDPE and nylon films. This strength could be increased with a decrease in plasticization levels and/or by the further incorporation of wax, as seen with the Shellhammer and Krochta (1997), the strength of casein-wax films could be comparable to some of the synthetic polymers. However, with an increase in wax content a large drop in elongation would likely occur. Lactic acid casein-based films without wax are shown to have slightly lower elongation properties than HDPE and EVOH. However, with the incorporation of wax we see these properties decrease drastically.

3.7.3 Heat Sealing

From preliminary testing it was found that no heat seal could be made at the melt temperatures of either wax, but a seal was formed around the melting temperatures around those of lactic acid casein and sorbitol, 100 and 110°C. A better heat seal was produced when sealing the bottom surfaces together. From the microstructure studies this showed a mostly protein-plasticizer structure. When sealing the top surfaces, in films containing wax, a weak seal that easily delaminated was produced. From this it can be concluded that either the lactic acid casein, the sorbitol, or a combination of both were responsible for the melt flow characteristics to obtain a good seal.

Although seal strengths were found to be rather weak, an acceptable heat seal

Table 18. Comparison of Lactic Acid Casein-Based Edible Films Mechanical Properties

Film Type[1]	**TS (MPa)**	**Elongation (%)**	**Test Conditions**	**Reference**
LAC(50)-S(50)	6.2	210	23°C, 50% RH	Present
LAC-S(35)-CR(15)	7.9	31	23°C, 50% RH	Present
LAC-S(35)-CN(15)	8.3	37	23°C, 50% RH	Present
WPI(60)-CN(40)	23	1.5	23°C, 50% RH	Shellhammer & Krochta (1997)
WPI(60)-CR(40)	17	1.0	23°C, 50% RH	Shellhammer & Krochta (1997)
LDPE	8.6-17	500	23°C, 50% RH	Salame (1986)
HDPE	17-35	300	23°C, 50% RH	Smith (1986)
EVOH	39-69	235-325	23°C, 50% RH	Foster (1986)
Nylon 6	69-83	400-500	23°C, 50% RH	Tubrity & Sibilia (1986)

[1] LAC - Lactic Acid Casein, S - Sorbitol, CR - Carnauba Wax, CN - Candelilla Wax, WPI - Whey Protein Isolate Solution, LDPE - Low Density Polyethylene, HDPE - High Density Polyethylene. Number in parenthesis is the percentage of that material in film (dry basis).

between the two film layers was able to be accomplished (Tables 19, 20, and 21). With an increasing seal temperature seal strengths tended to increase. Below a temperature of 93°C a good seal was not formed, and at temperatures greater than 121°C degradation of the films would start to occur, especially with a 4 second dwell time. This degradation did occur at 121°C in films without wax. Degradation in this study was noted as excessive burning/melting of the film, usually resulting in it sticking to the heat platens. These results correspond to DSC results where we observe film degradation beginning to occur around 120°C.

Seal strength also tended to increase with an increasing dwell time, from 2 to 4 seconds. This allowed the heat to penetrate further into the film thickness, creating more flow and interaction at the interfaces. Seal pressure, 40 or 60 psi, did not produce differences in the overall strength of the films.

A good seal, where a break in the seal would occur rather than delamination, was found to occur with a seal strength at around 0.6 MPa or greater. SEM pictures taken of films with strengths higher than this showed that good adhesion between the two film. layers had occurred (Figures 26, 27, and 28). This was concluded because a clear line of separation between the two film layers is difficult to differentiate, that is the two films layers appeared to have melted into one film.

Table 19. Seal Strength of Lactic Acid Casein (50%)-Sorbitol (50%) Edible Films

	Seal Strength (MPa) Seal Parameters			
Seal Temparature (°C)	**2sec-40psi**	**2sec-60psi**	**4sec-40psi**	**4sec-60psi**
200	0.22 ± 0.13^{a}	0.38 ± 0.29^{ca}	0.99 ± 0.64^{a}	0.66 ± 0.15^{a}
212	0.24 ± 0.17^{a}	0.67 ± 0.19^{a}	0.71 ± 0.31^{a}	0.62 ± 0.19^{a}
225	0.90 ± 0.54^{ab}	0.43 ± 0.18^{a}	1.33 ± 0.52^{a}	0.94 ± 0.54^{a}
250	1.37 ± 0.32^{b}	$1.81 \pm .34^{b}$	-	-

^{a,b} Different letter columnwise denotes significant difference ($p < 0.05$)

Table 20. Seal Strength of Lactic Acid Casein (50%)-Sorbitol (35%)-Carnauba Wax (15%) Edible Films

	Seal Strength (MPa) **Seal Parameters**			
Seal Temparature (°C)	**2sec-40psi**	**2sec-60psi**	**4sec-40psi**	**4sec-60psi**
200	0.28 ± 0.05^{a}	0.37 ± 0.12^{a}	0.80 ± 0.43^{ab}	0.70 ± 0.15^{a}
212	0.43 ± 0.09^{a}	0.31 ± 0.09^{a}	0.61 ± 0.10^{b}	0.79 ± 0.35^{a}
225	0.98 ± 0.43^{a}	0.57 ± 0.33^{a}	1.23 ± 0.14^{a}	0.79 ± 0.39^{a}
250	0.68 ± 0.30^{a}	1.29 ± 0.62^{b}	1.19 ± 0.36^{a}	1.63 ± 0.69^{a}

^{a,b} **Different letter columnwise denotes significant difference ($p < 0.05$)**

Table 21. Seal Strength of Lactic Acid Casein (50%)-Sorbitol (35%)-Carnauba Wax (15%) Edible Films

	Seal Strength (MPa) **Seal Parameters**			
Seal Temparature (°C)	**2sec-40psi**	**2sec-60psi**	**4sec-40psi**	**4sec-60psi**
200	0.13 ± 0.02^{a}	0.21 ± 0.08^{a}	0.65 ± 0.50^{a}	0.60 ± 0.40^{a}
212	0.43 ± 0.26^{ab}	0.22 ± 0.08^{a}	1.58 ± 0.80^{ab}	1.89 ± 0.72^{a}
225	1.23 ± 0.63^{b}	1.14 ± 0.74^{a}	1.53 ± 0.52^{ab}	1.90 ± 0.86^{a}
250	0.68 ± 0.30^{ab}	1.19 ± 0.85^{b}	2.47 ± 0.18^{b}	1.01 ± 0.43^{a}

[a,b] Different letter columnwise denotes significant difference ($p < 0.05$)

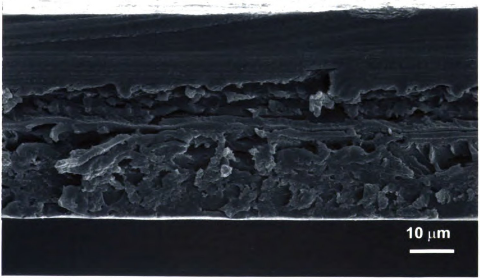


Figure 26. SEM of the Cross Sectional Surface of the Seal Area of Lactic Acid Casein (50%)-Sorbitol (50%) Edible Films

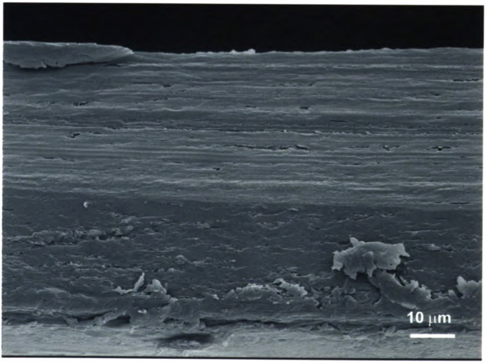


Figure 27. SEM of the Cross Sectional Surface of the Seal Area of Lactic Acid Casein (50%)-Sorbitol (35%)-Carnauba Wax (15%) Edible Films

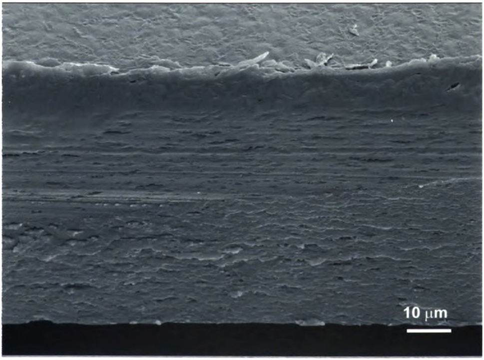


Figure 28. SEM of the Cross Sectional Surface of the Seal Area of Lactic Acid Casein (50%)-Sorbitol (35%)-Candelilla Wax Edible Films

4.0 CONCLUSIONS

4.1 Film Development and Characterization

1. Films with wax incorporated into them were less transparent than films without wax, but were still smooth and flexible.
2. Wax concentrations above 15% (on a primary component basis) could not be incorporated into films containing 50% lactic acid casein powder and sorbitol.
3. Films produced were found to be continuous and homogeneous.
4. Partial phase separation is believed to have occurred, due to a layering structure that was found in films containing wax, with the wax being found mainly at the top surface, and the presence of two endothermic peaks from thermal studies.
5. Moisture sorption isotherm data correlated well with theoretical values from the GAB model for lactic acid casein (50%)-sorbitol (50%) edible films.

4.2 Water Vapor Permeability

1. WVP significantly decreased ($p < 0.05$) as wax content (carnauba or candelilla wax) was increased from 5 to 15% in lactic acid casein (50%)-sorbitol-wax edible films at both 50 and 70% RH.
2. WVP of lactic acid casein (50%)-sorbitol-carnauba wax edible films were not significantly different ($p < 0.05$) at 50% RH then these films with no wax incorporated, while WVP was significantly higher ($p < 0.05$) at 70% RH.
3. WVP of lactic acid casein (50%)-sorbitol-candelilla wax edible films were significantly lower ($p < 0.05$) then these films with no wax incorporated at both 50 and 70% RH.

4. Lactic acid casein (50%)-sorbitol edible films made with candelilla wax had a significantly lower ($p < 0.05$) WVP then films made with carnauba wax, at both 50 and 70% RH and equal wax content.
5. WVP of lactic acid casein (50%)-sorbitol edible films increased as RH increased (from 50 to 70%), with and without wax (carnauba and candelilla wax, 5 to 15% wax) added.
6. Lactic acid casein (50%)-sorbitol edible films were found to be poor water barriers, with or without wax (carnauba or candelilla wax) incorporated compared to synthetic and wax polymers.

4.3 Oxygen Permeability

1. There was no significant change ($p < 0.05$) in OP in lactic acid casein (50%)-sorbitol (50%) edible films with the addition of wax, carnauba or candelilla wax, or as wax content increased from 5 to 15% (dry basis), with protein content constant.
2. There was no significant difference ($p < 0.05$) in OP between lactic acid casein-sorbitol edible films with carnauba wax compared to those with candelilla wax.
3. OP in lactic acid casein(50%)-sorbitol(50%) and lactic acid casein (50%)-sorbitol (35%)-wax (15%) edible films, carnauba or candelilla wax, tended to decrease as RH increased to 35%, followed by a significant ($p < 0.05$), sharp increase at 70% RH.
4. Lactic acid casein(50%)-sorbitol(50%) and lactic acid casein (50%)-sorbitol (35%)-wax edible films, carnauba or candelilla wax, were found to be good oxygen barriers as compared to synthetic polymers.

4.4 Mechanical Properties

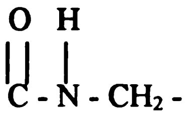
1. The addition of wax, carnauba or candelilla wax, significantly increases ($p < 0.05$) TS and significantly decreases elongation in lactic acid casein (50%)-sorbitol edible films.
2. TS significantly increases ($p < 0.05$) and elongation significantly decreases as wax content, carnauba or candelilla wax, increases in lactic acid casein (50%)-sorbitol edible films.
3. There is no significant difference ($p < 0.05$) in tensile or elongation properties between lactic acid casein (50%)-sorbitol edible films with carnauba or candelilla wax added to them.
4. A significant decrease ($p < 0.05$) in TS and a significant increase in elongation were observed in lactic acid (50%)-sorbitol-wax edible films when RH was increased from 50 to 75%.
5. Tensile and elongation properties of lactic acid casein (50%)-sorbitol-wax edible films are lower than those of synthetic polymers.
6. A good heat seal, although weak, of lactic acid casein-based edible films was obtained in the melting range near that of lactic acid casein and sorbitol, between 100 and 110°C (212 and 230°F).

5.0 APPENDICES

5.1 APPENDIX A

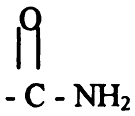
Appendix A. Structures of Various Compounds Found in Lactic Acid Casein-Sorbitol Edible Films

A1. Peptide



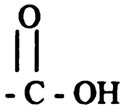
$$\overset{\overset{\displaystyle O}{\|}}{C}-\overset{\overset{\displaystyle H}{|}}{N}-CH_2-$$

A2. Amido



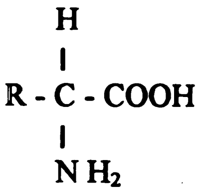
$$-\overset{\overset{\displaystyle O}{\|}}{C}-NH_2$$

A3. Carboxyl



$$-\overset{\overset{\displaystyle O}{\|}}{C}-OH$$

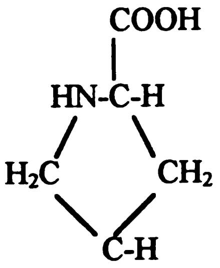
A4. Amino Acid



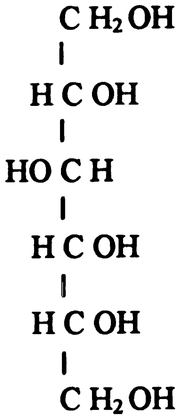
$$R-\underset{\underset{\displaystyle NH_2}{|}}{\overset{\overset{\displaystyle H}{|}}{C}}-COOH$$

R = side chain of variable composition

A5. Proline



A6. Sorbitol



$$\begin{array}{c} CH_2OH \\ | \\ HCOH \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

5.2 APPENDIX B

Appendix B. Amino Acid Composition of Casein Fractions

	Casein Type						
Amino Acid	α_{s1}-	α_{s2}-	β-	γ_1-	γ_2-	γ_3-	κ-
½ Cysteine	0	2	0	0	0	0	2
Alanine	9	8	5	5	2	2	15
Argenine	6	6	4	2	2	2	5
Asn	8	14	5	3	1	1	7
Aspartic Acid	7	4	4	4	2	2	4
Gln	15	15	21	21	11	11	14
Glutamic Acid	24	25	18	11	4	4	12
Glycine	9	2	5	4	2	2	2
Histidine	5	3	5	5	4	3	3
Isoleucine	11	11	10	7	3	3	13
Leucine	17	13	22	19	14	14	8
Lysine	14	24	11	10	4	3	9
Methionine	5	4	6	6	4	4	2
Phenylalanine	8	6	9	9	5	5	4
Proline	17	10	35	34	21	21	20
Serine	16	17	16	11	7	7	13
Threonine	5	15	9	8	4	4	14
Tryptophan	2	2	1	1	1	1	1
Tyrosine	10	12	4	4	3	3	9
Valine	11	14	19	17	10	10	11
Total	**199**	**207**	**209**	**181**	**104**	**102**	**169**

6.0 REFERENCES

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