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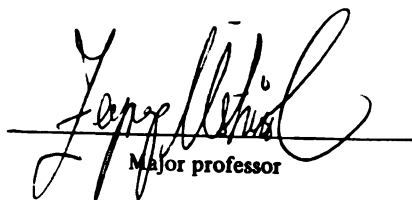
FORMULATION AND APPLICATION OF MILK
PROTEIN-BASED EDIBLE COATINGS

presented by

Julie S. Hazard

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M.S. degree in Food Science


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**FORMULATION AND APPLICATION OF MILK PROTEIN-BASED EDIBLE
COATINGS**

By

Julie S. Hazard

A THESIS

**Submitted to
Michigan State University
In partial fulfillment of the requirements
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ABSTRACT

FORMULATION AND APPLICATION OF MILK PROTEIN-BASED EDIBLE COATINGS

By

Julie S. Hazard

Milk protein-based edible coatings were formulated using whey protein isolate (WPI) or lactic acid casein (CAS), and sorbitol with or without carnauba wax or butter fat. The coatings were applied to the cut flesh of two cultivars of apples (Granny Smith and Ida Red). The coated apple halves were stored for 3 days at 4°C, 80% RH. The degree of browning of apple flesh, moisture loss of the cut apples, and water vapor resistance (WVR) of the coatings were evaluated by measuring the difference in color of apple flesh using a colorimeter, by determining the difference in weight, and mathematically using a modified Fick's equation, respectively. The coated apple halves were also evaluated (as described above) over 14 days of refrigerated storage and for degree of browning and acceptability using a trained visual panel.

WPI/lipid-based coatings were effective ($p < 0.05$) in retarding browning of cut Granny Smith apples. WPI-based coatings containing 1.5% lipid, were also effective ($p < 0.05$) in reducing moisture loss. Thus, these coatings had higher ($p < 0.05$) WVR. WPI/lipid coatings also were most effective in extending the shelf life of cut apples during refrigerated storage. Overall, CAS-based coatings were not as effective as WPI coatings. These WPI-based coatings could be used on cut Granny Smith apples on salad bars or for catering purposes to preserve the quality of minimally processed apples for two days if stored in LDPE packaging overnight.

Dedicated to my mother, Jane Nugent, who raised me to be independent and to follow my dreams; my grandparents, George and Lorraine Nugent, and my sisters Jennifer and Janine for all of their love and support. I also dedicate this achievement to my grandparents, John and Susan Hazard; and my father, Bill Hazard, for their love and financial support. I could not have completed this without all the support.

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INTRODUCTION

Edible coatings could reduce waste, enhance a food's organoleptic, mechanical and nutritional properties, and be used as a vehicle for supplying decorative effects, aroma, flavoring, and antimicrobial agents (Debeaufort and Voilley, 1994). By acting as barriers to moisture, gas and oil migration, coatings could provide protection to pieces or portions of foods. Additionally, they could be used as a barrier between food and the environment, or between two heterogeneous food components (Guilbert, 1986; Kester and Fennema, 1989a; Debeaufort et al., 1998). Coatings could form barriers against microbial contamination, and improve structural integrity and handling properties. The important characteristics and specific application depends on the product being coated and its primary form of deterioration. Coatings would also make it possible to use lower quality, less expensive, single-layer, synthetic materials for the primary packaging by complementing their purpose in managing the quality and stability of foods.

Recycleability of the packaging material would also be improved (Kester and Fennema, 1986; Donhowe and Fennema, 1994; Guilbert and Biquet, 1996; Mate and Krochta, 1996, Mate et al., 1996).

Edible coatings have been used for centuries to preserve food quality. In the twelfth and thirteenth centuries, the Chinese coated fresh lemons and oranges with wax to slow ripening and moisture loss. This coating was later found to inhibit respiration and induce fermentation (Hardenburg, 1967). In sixteenth century England, a process called larding was used to decrease the rate of moisture loss and shrinkage of meat (Labuza and Contreras-Medellin, 1981; Kester and Fennema, 1986). In the late 1800's, meat was

often coated with a gelatin film (Labuza and Contreras-Medellin, 1981; Kester and Fennema, 1986). Yuba, an edible film from the skin that forms on the surface of boiled soy milk, traditionally was used in Asia to preserve and enhance the appearance of various foods (Gennadios et al., 1993; Guilbert and Biquet, 1996). In 1954, gelatin capsules were introduced on an industrial scale to coat pharmaceutical tablets (Munden et al., 1954). In the food industry, chocolate and sugar coatings have been applied to nuts, dried fruits, candies, and cakes. Wax coatings have been applied to whole fruits and vegetables such as apples, avocados, citrus, cucumbers, eggplant, sweet peppers and tomatoes (Hagenmaier and Shaw, 1992) to preserve freshness and quality (Kroger and Igoe, 1971).

In this research, milk protein-based edible coatings were developed for possible application to minimally processed apples. Minimal processing such as cutting, peeling, or slicing has been known to cause undesirable changes in fruits and vegetables. Cellular integrity is lost due to destruction of enzyme and substrate compartmentation, which may result in enzymatic browning, secondary metabolite formation, and other deteriorative changes. Moisture loss, senescence, and development of off-flavors also may be accelerated. Applying edible coatings may be a method used to delay the occurrence of these processes or to decrease the extent in which they happen (Ghaouth et al., 1991; Burns, 1995; Brancoli et al., 1997). Both casein and whey proteins were used in coating development. The coatings developed were evaluated for their moisture and oxygen barrier properties, their ability to reduce the browning of the cut apples and extend their shelf life during refrigerated storage.

LITERATURE REVIEW

The consumer trend for the 1990's was convenience and fresh-like quality using more natural ingredients. Consumers wanted variety, added value, and fresh alternatives (Burns, 1995; Sloan, 1996; Hoover, 1997). Reyes (1996) reported that the current market for minimally processed fruits and vegetables in the US was valued at \$6 billion and was predicted to increase to \$20 billion by 2001. To answer consumer requests for more convenience foods, the food industry introduced products like prepackaged single-serving fresh-cut salad, and Stockpot's™ fresh refrigerated soup line. Fruit and vegetable snack packs for kids such as grapes, broccoli, baby carrots with dip, and breadsticks have also been introduced. Consumer acceptance of these products has been high; and Hoag (1995) reported that in a recent survey 52% of households would purchase fruits more frequently if they were more convenient to prepare.

The newfound popularity of table grapes in foodservice and as a healthy snack has prompted the grape industry to look into snack-size packaging (Burfield, 1998). Presliced, pitted, prepackaged watermelon was also being considered in foodservice for year round availability (Offner, 1998). Hoag (1995) predicted that the market for minimally processed fruit would be similar to that of ready to eat salads and vegetables. Precut melons, pineapple, and strawberries have been available for a relatively short time and have proven successful.

Snack foods such as carrot sticks have been packaged in modified (MA) or controlled (CA) atmospheres to control moisture loss, ripening, and microbial quality. However, carrots formed the white blush which consumers view as an indication of

inferior quality. The demand for presliced apples is expected to increase rapidly once an acceptable product is developed (Hoag, 1995). The application of an edible coating in addition to the MA packaging already used for these snack packs could improve the overall quality of the product, making it more marketable to the public. If edible coatings succeed in protecting the quality of minimally processed produce, a whole new market worth billions of dollars would open up for minimally processed fresh produce (Stephens, 1994).

1.1. Materials suitable for coating foods and their formation process

Edible coatings have been defined as edible layers that can be formed on the surface of food products, providing a semi-permeable barrier to gases and water vapor (Conca and Yang, 1993; Baldwin, 1994; Brancoli et al., 1997). Researchers reported that coating formulation requires at least one high molecular weight polymer or compound that is capable of forming a continuous, cohesive structural matrix and sometimes a solvent medium such as water or ethanol, which must be dried or evaporated. Coatings have required long-chain polymeric structures to yield coating matrices with appropriate cohesive strength when dried.

1.1.1 Properties of coating components

Coatings that were applied directly to a product had both cohesion between the molecules of the coating material and adhesion between the coating and the support structure. The degree of cohesion depended on the chemical structure of the polymer, the solvent used, the thickness of the coating, and the plasticizer and/or cross-linker if present

(Banker, 1966). The degree of cohesion of coatings also depended on the temperature of application and the speed at which the solvent was evaporated if used. Cohesion could be enhanced by an increase in polymer chain length and polarity (Guilbert, 1986; Kester and Fennema, 1986; Cuq et al., 1995; Debeaufort and Voilley, 1994; Guilbert and Biquet, 1996). Banker (1966) found that the most cohesive coatings were formed with the application of a warm coating solution, and that cohesion increased with coating thickness but decreased with excessive drying temperature and inadequate drying time.

1.1.2. Categories of high molecular weight polymers that can form edible coatings

Two categories of high molecular weight polymers used to form edible coatings are hydrocolloids and lipids. Composite coatings have been produced by incorporating hydrocolloids and lipids either as a layered coating or an emulsion (Donhowe and Fennema, 1994; Debeaufort et al., 1998). Composite coatings were effective when multiple barrier properties were desired because the second component incorporated the properties that the other component lacks (Schultz et al., 1949; Kamper and Fennema, 1984a, b; Debeaufort et al., 1998).

1.1.2.1. **Hydrocolloids.**

Researchers reported that hydrocolloids such as proteins and polysaccharides are structurally durable and generally have good lipid and gas barrier properties, but they were not recommended to control moisture migration. Studies have shown that protein or polysaccharide-based coatings had strong mechanical properties, could improve the

structural integrity of some products, and can adhere to hydrophilic surfaces such as the flesh of sliced fruit (Kester and Fennema, 1986; Gennadios and Weller, 1990).

1.1.2.1.1. Protein-based edible coating materials and their properties. Proteins were effective coating formers that could add a nutritional component and readily adhere to hydrophilic surfaces such as cut fruit (Gennadios and Weller, 1990). Proteins denatured more readily than other types of polymeric material and most protein-based coatings were poor moisture barriers. However, they have been shown to be effective as oxygen barriers (Conca and Yang, 1993; Anon., 1990; Gennadios and Weller, 1990).

1.1.2.1.1a. *Corn zein* has been extracted from maize and is soluble in 70% ethanol or prolamine solutions. It has been reported to contain a high percentage of nonpolar hydrophobic amino acids and can form a tough, glossy, greaseproof coating with efficient gas barrier properties (Guilbert, 1986). Corn zein has been used for coating confectionery products, nuts, and pharmaceuticals, and has been useful for microencapsulation (Gennadios and Weller, 1990). Coatings made with corn zein may have an objectionable color, which could be made colorless using decolorization materials (Guilbert and Biquet, 1996). Corn zein coatings were reported to be transparent and tasteless (Park et al., 1994c). Cozeen™ (Zumbro Inc., Hatfield, MN) has been a commercial edible coating that contains corn zein as a major component (Park et al., 1994c).

1.1.2.1.1b. **Wheat gluten**, a globular protein, was reported to be responsible for the cohesive structure and elasticity of wheat dough. Kneading wheat dough and then washing away the starch produces gluten. Coatings were formed by the mechanism of simple coacervation (Gontard et al., 1992) where disulfide bonds were reduced and cleaved by mixing the gluten in an alkaline solution with a reducing agent. A coating was made when new disulfide bonds were formed by re-oxidation in air. Wheat gluten coatings had low oxygen and carbon dioxide permeability and have been used as flavor and color carriers in baking. Wheat gluten also has been used as a binder in nut salting (Gennadios and Weller, 1990; Gontard et al., 1992). The coatings had objectionable characteristics including a slight yellow color, chewy texture and alkaline taste.

1.1.2.1.1c. **Gelatin** is a protein derived from collagen. Gelatin coatings have been formed by the ionic cross-linking of the amino acids within the gelatin and were clear, and transparent (Guilbert and Biquet, 1996). Gelling has been reported to be thermally reversible and therefore gelatin had poor moisture barrier properties. However, acid treatment has been reported to improve these properties (Kester and Fennema, 1986). Gelatin was an excellent oxygen barrier and has been used to coat pharmaceutical products (Conca and Yang, 1993) and as packaging of dry products (Guilbert, 1986).

1.1.2.1.1d. **Soy protein** coatings have been formed on the surface of heated soymilk. Soy protein coatings made using traditional techniques (boiling soymilk and removing the skin that formed as a result) were water-resistant. Soy protein coatings, which were made with isolated soy protein, were poor water barriers. Forming coatings

with isolated proteins yielded mechanically stronger coatings (Guilbert, 1986). Soy has been commonly used as a nut coating, a pre-dusting material for fried chicken, an encapsulating material and as a coating for meat (Gennadios and Weller, 1991).

1.1.2.1.2. Polysaccharide-based edible coating materials and their properties.

Polysaccharides such as alginate, carrageenan, pectin, starch, and cellulose have been studied as potential coating materials. These polymers have been reported to be hydrophilic in nature, therefore the coatings were expected to have minimal water barrier properties. Polysaccharides have been reported to have better water barrier properties as a gel-like coating, which acted as a sacrificing agent rather than a barrier. Sacrificing agents have been reported to absorb moisture from the air and product, which would keep the moisture within coated product system. Researchers report that polysaccharides can adhere to cut surfaces of fruits and vegetables which may provide an effective barrier to oxygen and other gasses (Banker, 1966; Kester and Fennema, 1986, Baldwin et al., 1995). The desirable properties that polysaccharide coatings could provide may increase shelf life without developing anaerobic conditions (Baldwin et al., 1995). The major mechanism of polysaccharide coating formation included the breakage of polymer segments and reformation of polymer chains into a gel or coating matrix. Hydrophilic and hydrogen bonding and/or electrolytic and ionic cross-linking needed to form the coating usually resulted from evaporation of a solvent (Guilbert, 1986; Kester and Fennema, 1986).

1.1.2.1.2a. *Alginate* has been extracted from brown seaweed and formed a gel when calcium ions are added to an aqueous solution of sodium alginate (Butler et al., 1996). Alginate-based coatings have been formed by evaporation, or electrolytic cross-linking (Kelco, 1976). Calcium gelation of sodium alginate can be achieved by the release of divalent calcium ions within an alginate solution, or by diffusing calcium ions into the alginate solution (Glicksman, 1983; Deasy, 1984). The mechanical properties of alginate coatings can be altered with a change in polyvalent cation concentration such as calcium ions. Also, mechanical properties can be modified by the rate of its addition, time of exposure, change in pH or temperature, or the addition of plasticizers or other additives. Alginate coatings had good oxygen barrier properties and poor water barrier properties. Alginate coatings have been applied to meat to protect against oxidation (Kester and Fennema, 1986).

1.1.2.1.2b. *Carrageenan* is a complex mixture of polysaccharides extracted from red seaweed. Heating and cooling of an aqueous solution of carrageenan formed a gel. Coatings formed from this gel were weak and appeared cloudy. Carrageenan has been mainly used as an emulsifier, thickener, stabilizer, gel and coating (Kester and Fennema, 1986; Dziezak, 1991; Baldwin et al., 1995). Adding sorbic acid to carrageenan coatings may decrease growth of surface microorganisms in intermediate moisture foods (Kester and Fennema, 1986; Dziezak, 1991).

1.1.2.1.2c. *Pectin* is a complex group of polysaccharides found in plants which when de-esterified yields low-methoxyl pectins. When low-methoxyl pectins were

dissolved in solution in the presence of calcium ions, they formed gels (Butler et al., 1996; McHugh et al., 1996). Aqueous solutions of low-methoxyl pectin were applied to the food surface, then a calcium solution was applied to form a gel (Kester and Fennema, 1986). Pectin-based coatings were poor water barriers, but could act as sacrificing agents to retard moisture (McHugh et al., 1996; Kester and Fennema, 1986). Fruit purees were used to form coatings due to their high pectin content. A fruit puree coating matrix was developed with pectic and cellulosic substances. The sugars in fruit puree acted as plasticizers. Fruit puree coatings were effective oxygen barriers for low to medium moisture foods such as baked goods, nuts and confections. They could also be good flavor, aroma and lipid barriers (McHugh et al., 1996).

1.1.2.1.2d. *Starch* has been composed of amylose and amylopectin. Modified starch containing 85% amylose has been used to form coatings. Heating aqueous solutions of starch, followed by drying formed coatings on foods. Starch coatings were transparent and were reported to be effective oxygen barriers, as well as semipermeable barriers to CO₂ (Jokey et al., 1967; Whistler and Daniels, 1985; Kester and Fennema, 1986). Partial etherification of starch with propylene oxide yields a hydroxypropylated derivative, which made coatings with poor water barrier properties and effective oxygen barriers. Starch coatings have been reported to be brittle, break easily, and be milky white in color. They usually had no objectionable odor (Conca and Yang, 1993).

1.1.2.1.2e. *Cellulose* has a tightly packed highly linear crystalline structure. Treatment with alkali made cellulose water soluble (Butler et al., 1996). The addition of

chloroacetic acid, methyl chloride, or propylene oxide yielded carboxymethylcellulose, methylcellulose, hydroxypropyl methylcellulose, or hydroxy propylcellulose which formed coatings with effective moisture barrier properties (Hagenmaier and Shaw, 1990). Cellulose coatings formed by thermal gelation. When heated, the aqueous solution of cellulose reached a critical temperature at which a 3-dimensional gel structure formed. The gel reverted back to a liquid form when cooled below a critical temperature (Whistler and Daniels, 1985). Methylcellulose and hydroxypropyl methylcellulose have been used to coat frozen fried foods such as french fries and onion rings to decrease oil absorption during cooking. Researchers report that hydroxypropyl cellulose is thermoplastic and can be extruded (Kester and Fennema, 1986). Cellulose-based coatings were reported to be tasteless, odorless, transparent and have acceptable texture and mouthfeel (Conca and Yang, 1993).

1.1.2.1.2f. *Chitosan* is a glucosamine produced by deacetylation of chitin, a naturally occurring biopolymer. Chitin has been harvested from the shells of crustaceans; however, it has also been produced from shellfish waste and fungal cell walls (Conca and Yang 1993). Chitosan formed durable coatings with a slightly yellow appearance. The water barrier properties of chitosan coating increased with an increase in degree of acetylation, and have been chemically treated to increase the water solubility of the coating. Chitosan coatings have been found to have oxygen barrier capabilities and inhibit growth of several fungi such as *Aspergillus niger*, *Rhizopus nigricans*, and *Alternaria alternata* (Hirano and Nagao, 1989), however it was a poor water barrier (Butler et al., 1996). Chitosan has been commercially used to clarify and purify water

and other beverages, as well as detoxifying hazardous waste. Chitosan has also been used in cosmetics due to its humectant and cationic characteristics, and to coat agricultural seeds to increase crop yields. It has also been used on strawberries to increase quality and shelf life (Ghoauth et al., 1991).

1.1.2.2. Lipids

1.1.2.2.1 Lipid-based edible coating materials and their properties. Lipid and acetoglyceride coatings have been formed by solidification of the molten lipid. Lipid coating materials have included paraffin wax, mineral oil, beeswax, carnauba wax, candelilla wax, acetylated monoglycerides, and resins. Lipids by themselves generally lacked structural integrity and durability and therefore were used in combination with a hydrocolloid coating in the form of an emulsion or as a bilayer coating (Kester and Fennema, 1986; Baldwin et al., 1997). Lipids were effective gas and water barriers. These barrier properties were dependent on lipid crystal orientation and packing, and on the ratio of hydrophilic to hydrophobic materials in the formulation (Baldwin et al., 1997). Waxes have been used extensively on whole fruits and vegetables (Hagenmaier and Shaw, 1990 & 1992) to add a gloss or shine, but they tended to crack or break loose from the fruit or vegetable during shipping. Waxes did not stick to hydrophilic moist surfaces, and they tended to cause anaerobic conditions at high storage temperatures (Baldwin et al., 1995).

1.1.2.2.1a. *Acetoglycerides* were reported to be glycerol monostearate acetylated with acetic anhydrides. Commercially it has been used as an additive or ingredient, and

can form a flexible wax-like solid from the molten state. The moisture barrier properties of acetylated monoglyceride coatings were much lower than most polysaccharide coatings, except ethyl or methylcellulose (Kester and Fennema, 1986). Researchers reported that acetoglycerides had objectionable flavor and odor, but has been used in conjunction with other coatings to improve the water barrier properties (Conca and Yang, 1993).

1.1.2.2.1b. *Waxes* had better moisture barrier properties than most other coating materials. Paraffin wax exhibited the best moisture barrier properties because of its long-chain saturated hydrocarbons and non-polar groups. Beeswax has been comprised of hydrophobic long-chain ester compounds, long-chain hydrocarbons, and long-chain fatty acids. Researchers reported that beeswax can keep antimicrobials at the food surface so they do not diffuse into the food. Fruits and vegetables have been commonly coated with wax to replace the natural wax lost due to washing. Waxes reduced respiration, water loss, and retarded senescence. Complete coverage, however, induced anaerobic respiration and increased the onset of senescence (Kester and Fennema, 1986).

1.2. Additives and their function in edible coatings

Guilbert (1986) reported that functional properties of coatings depend on the formulation and concentration of the basic components, pH, denaturation temperature, heating temperature, addition of additives, application technique, and the environmental conditions in which the coating was applied. Additives can be incorporated into edible coatings to alter sensory, nutritional, physical and mechanical properties. Additives such

as plasticizers, antimicrobials, antioxidants, cross-linking agents and lipids can be incorporated to change the mechanical and physical properties of a coating (Donhowe and Fennema, 1994; Cuq et al., 1995; Debeaufort and Voilley, 1994). The effect of an additive on coating properties has been found to depend on its chemical structure, concentration and dispersion in the coating, and its interaction with the polymer (Okhamafe and York, 1984).

1.2.1. Plasticizers

Researchers reported that plasticizers are compounds that, when added to another material, can alter its mechanical and physical properties (Banker, 1966; Guilbert and Biquet, 1996). These have included polyhydric alcohols such as sorbitol, sucrose, propylene glycol, and glycerol, waxes, and oils, which have been reported to reduce internal bonding and increase intermolecular spacing (Lieberman and Gilbert, 1973). They can be categorized as internal or external plasticizers. Internal plasticizers have been reported to modify the chemical structure of the polymer by disrupting the bonding of the polymer and by copolymerization, hydrogenation, or transesterification (Donhowe and Fennema, 1994). External plasticizers, which have been most used for protein-based edible coatings, are additives which have been reported to modify the physical properties and barrier properties of the coating polymer (Banker, 1966). These added flexibility and elongation to polymeric substances and prevented cracking and improved coating performance by weakening the intermolecular forces between the polymer chains (Chuah et al., 1983; Andres, 1984; Gennadios and Weller, 1990; Debeaufort and Voilley, 1994). A plasticizer should be compatible with, and have similar solubility to the polymer and

the solvent used, and be permanently present in the polymer-solvent system (Guilbert and Biquet, 1996).

1.2.2 Antimicrobials

Antimicrobial agents such as fungicides, benzoic acid, and sorbic acid can be added to retard yeast, mold, and bacterial growth. Using edible coatings as a vehicle allowed application of antimicrobials onto the food surface where most deterioration begins (Kester and Fennema, 1986, Baldwin et al., 1995) without destruction of the foods integrity (Guilbert, 1986). It also allowed for the addition of lower quantities of antimicrobial agents because they are maintained on the surface. Antimicrobial agents were limited in their effectiveness on a food surface because they eventually diffused into the food (Baldwin et al., 1995). However, Guilbert and Biquet (1996) reported that edible coatings might delay the diffusion of the antimicrobial into the food, and may increase the shelf life of the food.

1.2.3. Antioxidants

Antioxidants can be added to a coating to slow oxidative rancidity, degradation and discoloration. Phenolic compounds such as BHA, BHT, TBHQ, tocopherols, and acids such as propyl gallate and ascorbic acid prohibited oxidation of lipids. Polyphenolic compounds acted with acidic substances such as citric acid, phosphoric acid, and ascorbic acid to create effective chelating agents which prevented browning (Stucky, 1972; Nisperos-Carriedo et al, 1988). Metal chelators such as EDTA are also added to slow oxidative rancidity (Baldwin et al., 1995).

1.3. Processing approaches and application techniques

1.3.1 Processing approaches utilized in forming edible coatings

The processing approaches used to form coatings from hydrocolloids are simple coacervation, complex coacervation, and thermal gelation and coagulation. Simple coacervation has involved the separation of a single hydrocolloid from an aqueous suspension or solution. A change in phase due to heating, evaporation or addition of solvents, altering pH or charge on the polymer, or addition of an electrolyte to cause salting out or cross-linking is also simple coacervation (Bankan, 1973; Kester and Fennema, 1986). Complex coacervation has involved the combination of two oppositely charged hydrocolloid solutions through the mechanism of charge neutralization, causing interaction and precipitation of the polymer complex (Glicksman, 1982; Kester and Fennema, 1986). Thermal gelation or heat coagulation has involved heating a macromolecule solution, which causes denaturation, gelling, or precipitation resulting in recrystallization, or the cooling of a warm hydrocolloid suspension to cause sol-gel transformation (Kester and Fennema, 1986; Donhowe and Fennema, 1994).

Lipid coatings are formed by solidifying a hot liquid, or by forming an emulsion with water or another organic solvent, which would then be evaporated. A lipid can also be applied in solution or applied as an oil-in-water emulsion with subsequent drying or evaporation of solvent (Kester and Fennema, 1986).

1.3.2. Application techniques

Many techniques can be used to apply edible coatings to a food product. Dipping was reported to be the simplest method (Hardenburg, 1967; Chen, 1995). Dipping has

been perfect for irregularly shaped products that require several applications, and formed a relatively uniform coating that covers the entire product. Dipping has been adequate for small quantities of food and has been used to coat products such as meat, fish, poultry, and whole fruits and vegetables (Donhowe and Fennema, 1994; Grant and Burns, 1994). Once the product is coated, it is dried in a drying apparatus or under ambient conditions. One problem with dipping is that the surface of the product must be completely dry before dipping so the coating solution does not become diluted (Grant and Burns, 1994).

Spraying has been the technique most widely used in industry. It has formed a thinner, more uniform coating than dipping, and has been more suitable for applying a coating on one side of a food, such as frozen pizza, where a barrier may be desirable between the crust and the moist sauce (Donhowe and Fennema, 1994). An entire product can be coated by spraying the product with a mist of coating material at a constant rate while being rotated so that all surfaces can be uniformly coated. Roller brushing in addition to spraying has been used for larger particulate objects such as citrus fruits (Grant and Burns, 1994; Chen, 1995). High-pressure spray applicators have also been used to apply edible coatings. However, the nozzles can become clogged or deliver excess coating. Air-atomizing systems also could be used, however they are more expensive (Grant and Burns, 1994).

Foaming has been less frequently used because it is difficult to form a uniform coating with this method (Chen, 1995). The coating to be used is foamed either by introducing compressed air into the applicator tank or by adding a foaming agent to the coating. The foam is then dropped onto the product as it moves over rollers and is spread

over the surface of the product by brushes or cloth flaps. Excess coating is removed and may be reused. The advantage of using a foamed emulsion is that it dried very quickly (Grant and Burns, 1994).

1.4. Uses of edible coatings

Coatings have been used for centuries to preserve the appearance and improve the shelf life of foods. In 1978, Wu and Salunkhe evaluated the affect of lecithin and hydroxylated lecithin coatings on light synthesis of glycoalkaloids and chlorophyll in potato tubers. These researchers found that the coatings significantly reduced the synthesis of chlorophyll and glycoalkaloids exposed to light, but did not effect the glycoalkaloids that already existed in the potatoes. The concentration of lecithin in the coating affected the extent to which the inhibition occurred such that increased lecithin concentration equaled increased inhibition.

In 1985, Banks conducted a similar study using a 3% solution of Pro-long™ (a commercial sucrose polyester/carboxymethylcellulose based coating). He coated King Edward potatoes with Pro-long™ and determined its effects on potato tuber greening. He compared the coated samples to samples placed in modified atmospheres (MA) of 15% CO₂/21% O₂ and 5% O₂, and uncoated controls. The coated and MA treatments reduced light induced greening. Coating caused small internal atmosphere changes, which in turn suppressed greening due to the lower chlorophyll content.

Mazza and Qi (1991) evaluated the performance of various coating products and calcium chloride on the control of after-cooking darkening in potatoes. These coatings were applied to water-blanchd potato strips. Treatment effectiveness was evaluated

using quantitative measurements of color change. Purified spray dried gum acacia (Spraygum), and gum acacia/gelatin coatings in combination with calcium chloride showed promise as inhibitors to after-cooking darkening of blanched potato strips.

Commercial Semperfresh™, a sucrose polyester coating, was evaluated for its effects on maturity of fruits during cold storage. Santerre et al. (1989) coated whole Golden Delicious and McIntosh apples with Semperfresh™ coating and determined its effects on skin color, firmness, and sensory evaluation. There was no significant affect on internal color of McIntosh or Golden Delicious apples; however, there were significant affects on external color over the storage period for McIntosh that could be attributed to changes in gas concentration. There was no external color affect for Golden Delicious apples. The coated apples maintained firmness and that increasing Semperfresh™ concentration decreased softening during storage. Semperfresh™ coating had no affect on internal soluble solids, pH, or titratable acidity of either apple variety. This could be due to the advanced maturity of the test apples. Taste panel triangle results showed no significant flavor or texture affects between coated and uncoated apples of both varieties.

Chai et al. (1991) also studied the influence of Semperfresh™ coatings on maturity parameters, ripening, and shelf life of Golden Delicious, Ida Red, and McIntosh apples during cold storage. Semperfresh™ coatings retarded apple ripening, and increased tissue firmness and titratable acidity, however soluble solids were not significantly affected. Semperfresh™ treatments did improve consumer acceptability for Golden Delicious and McIntosh apples, but not for Ida Red apples. These results did not agree with those of Santerre et al. (1989).

Park et al. (1994a) used Semperfresh™ to coat whole Red Delicious, Rome Beauty, and Arkansas Black and reported the effects of coating thickness on gas transmission rates. As the concentration of sucrose polyester increased, coating thickness increased. They also found that the thicker coatings had better barriers to gases. All coatings applied to Red Delicious were thicker than the same coatings applied to Rome Beauty and Arkansas Black. They concluded that this may have been caused by differences in surface characteristic of the apples. The oxygen permeabilities of the Semperfresh™ coatings were higher than most protein-based coatings, but were similar to those of cellulose coatings. The water vapor permeabilities were significantly better than corn zein-based or cellulose-based coatings.

As convenience foods such as pizza and pre-prepared ice cream cones became more popular, moisture migration of a high moisture food to a low moisture food, became a shelf life concern. Kester and Fennema (1989a) studied the effects of an edible lipid cellulose ether composite film as on internal moisture migration between a piece of white sandwich bread and tomato sauce in frozen storage. The film retarded moisture migration effectively during storage, and the stabilization of moisture gradients measured after cooking was maintained significantly better with the coating than without. However these coatings did not completely curtail the transmission of water. This water transmission may have been an effect of freeze-thaw stress. Bread sogginess increased significantly over time with or without the coating, but the coated bread had significantly less sogginess than the uncoated bread. A waxy mouth feel and bitter flavor was noted in the coated samples as determined by a trained sensory panel.

Researchers studied the effects of the addition of antioxidants to coatings to extend shelf life of fruits and vegetables. Nisperos-Carriedo et al. (1991) developed an edible composite coating containing wax and ascorbic acid/calcium chloride or ascorbic acid/ EDTA to extend the shelf life and retard ripening of whole fresh fruits such as tomatoes, mangoes and bananas. They also tried these coatings on mushrooms to prevent enzymatic browning. The coatings delayed ripening in some climacteric fruits, and the effects could be improved by altering the coating to fit the specific requirements of the food being coated. They also showed a decrease in the rate of browning of mushrooms and found that the anti-browning property was improved with the addition of an antioxidant (ascorbic acid) and a chelator (calcium disodium EDTA).

Ghaouth et al. (1991) examined the effect of chitosan, a polysaccharide coating, had on controlling decay of fresh strawberries compared to fungicide treatments. After 21 days, 10% of the chitosan-coated strawberries were decayed as compared to 52% of the uncoated fruit. Compared to the fungicide treatment, chitosan coatings significantly reduced decay, and coated fruit was more firm, and had higher titratable acidity. The chitosan coating had an immediate effect on respiration, however a reduction in respiration rate was not evident until the fourth day of storage of the fresh strawberries.

Lipid oxidation and subsequent quality and shelf life deterioration of confectionery products became a concern. Three hydrocolloid/sweetener based coatings developed and tested by Brake and Fennema (1993) had suitable water activity, viscosity and adhesion to chocolate. The first coating contained Amalean II (a pregelatinized, hydroxypropylated amylose starch), sucrose, fructose, dextrose, high fructose corn syrup, and water. The other two coatings contained BB Rapid Set (a high methoxy pectin),

Acacia gum, sucrose, fructose, dextrose, high fructose corn syrup, and water. These coatings were placed between a chocolate layer and a lipid containing peanut butter center. Amalean II-based coating and the BB Rapid Set coating containing the lower concentration of pectin were effective barriers to lipid migration. The Amalean II-based coating scored significantly lower than the controls with no coating for all sensory attributes tested, whereas the BB Rapid Set coating did not differ significantly from the controls for first-bite characteristics, post-chewing texture, or overall acceptance, but did score significantly lower for visual properties.

Stuchell and Krochta (1995) used whey protein isolate/acetylated monoglyceride coatings to test their effectiveness against moisture loss and lipid oxidation of frozen king salmon. They found that acetylated monoglyceride coatings alone or with the added layer of whey protein reduced moisture loss by 42% to 65% in the first 3 weeks of storage. The coatings containing acetylated monoglyceride or oversprayed with antioxidant delayed lipid oxidation as determined by and reduced peroxide values. They reported no significant differences in the effectiveness of the different treatments.

The onset of lipid oxidation and rancidity has been a major shelf life issue for packaged dry-roasted peanuts. Mate and Krochta (1996) coated dry-roasted peanuts with whey protein isolate (11% w/w)/glycerol coatings in ratios of 1:1 and 3:2 to determine the effects of the coatings on the oxygen uptake of dry-roasted peanuts. These coatings were stored at 29 or 37°C, 21% or 53% RH. The oxygen uptake was evaluated and compared to the uncoated controls. The researchers found it was necessary to increase glycerol content when storing peanuts in lower RH in order to preserve the mechanical properties of the coatings. The coatings were more effective when peanuts were stored at

a lower temperature and RH. The whey protein isolate coatings delayed oxygen uptake of dry-roasted peanuts. The extent of delay depended on the combination of coating thickness, plasticizer content, and RH of the storage area. They concluded that a thicker coating stored at a lower RH resulted in more effective coatings, and that its effectiveness relied on oxygen-barrier properties of the coatings.

Mate et al. (1996) continued the above research and studied thickness and RH effects of whey protein isolate/glycerol coatings on the rancidity of dry-roasted peanuts. Whey protein isolate/glycerol coatings delayed oxidative rancidity of dry-roasted peanuts. They found that the coated peanuts took twice as long to reach the same peroxide value (PV) as uncoated peanuts and the final PV of the coated peanuts was lower than the uncoated peanuts. A thicker, more continuous coating and storage at a lower RH resulted in more effective coatings.

As minimally processed, ready to eat carrots became more and more popular, researchers studied the possibility of using edible coatings to decrease white blush formation and improve the surface appearance of these packaged carrots. Avena-Bustillos et al., (1993) studied how sodium caseinate/stearic acid emulsions affected white blush formation on minimally processed carrots. Coating formulations consisted of 1 to 3% total solids emulsions of sodium caseinate and stearic acid. These coatings were sprayed onto peeled carrots and then were air-dried. Coatings were evaluated for water vapor resistance (WVR), whitish index, and sensory quality. All coating formulations except the one containing 2% sodium caseinate significantly increased WVR compared to the uncoated control. Coatings did not reduce white blush formation consistently during storage as determined by the whitish index; therefore the significant difference

between samples was not consistent from day to day. Sensory analysis concluded that coated samples were consistently visually ranked lower in white blush formation than the uncoated control. They hypothesized that hydrophilic coatings help to moisturize the carrot surface, reducing white blush formation and decreasing the whitish index.

Sargent et al. (1994) determined the effectiveness of carboxymethylcellulose (CMC) edible coatings on reduction of white blush formation, storage life and quality of peeled carrots over a 30-day period. The coatings included a 1% CMC coating, 1% CMC/parabens coating, 2% CMC/parabens coating, 1% CMC/parabens/propionic acid coating and 1% CMC/citric acid/propionic acid coatings. The coatings were stored in microperforated plastic bags without a drying period and consequently remained tacky for up to 26 days of storage. The coatings that dried faster during storage decreased the appearance of surface whitening. Coated carrots were reported to have significantly less surface drying and more acceptable appearance compared to the uncoated controls throughout the 30-day storage period. It was also noted that the coating containing propionic acid and the 1% CMC coating with no preservative had a noticeably improved appearance. There were no differences in moisture content, which ranged from 88.9% to 89.8% during storage. There were no differences in decay of the samples over time as there were microbial agents added to all coating solutions. Coatings containing added acidulant or higher viscosities performed the best against white blush formation.

Avena-Bustillos et al. (1994a) coated minimally processed carrot sticks with calcium caseinate and sodium caseinate-based coatings containing beeswax, stearic acid, or acetylated monoglyceride. They concluded that white blush formation was a result of

dehydration and that the RH level affected the rate of white blush formation. All of the coatings reduced white blush, respiration rate, and increased water vapor resistance.

Howard and Dewi (1995) studied the effect of the application rate of a Nature Seal™ 1000 spray, an edible cellulose-based coating, on the sensory, microbiological, and chemical qualities of mini-peeled carrots. All spray application rates significantly reduced the rate of surface discoloration as determined by sensory analysis. The least discoloration occurred on samples treated with the highest spray rate of coating application. No significant differences were found in the APC, yeast, mold, or lactobacilli counts compared to a control. They reported that the higher application rate increased the degree of slipperiness on the carrot surface. Sensory scores for fresh carrot flavor, aroma, and overall acceptability were higher for the coated sample than the uncoated controls.

Howard and Dewi (1996) applied Nature Seal™ cellulose based edible coatings to mini-peeled carrots to evaluate coating effects on flavor, aroma, sweetness, appearance, slipperiness, carotene and terpenoid content. Coated carrots scored lower than the control for white surface discoloration, and higher for orange color intensity and appearance. They attributed the prevention of white discoloration to the retardation of moisture loss. Coating treatment did not affect carrot flavor, aroma, or sweetness. Carotene and terpenoid content decreased in the coated carrots, but this decrease did not differ from the uncoated control. They suggested that this lack of effect could have been a result of the coating's poor oxygen-barrier properties, enzymatic degradation, or oxidation of carotenes after peeling.

Zucchini has traditionally been coated with wax to decrease the moisture loss that occurs during storage. Wax coatings, however have been found to create anaerobic conditions within the food (Hardenburg, 1967). Avena-Bustillos et al. (1994b) coated zucchini with Semperfresh™ coating, and Alanate 310™ (calcium caseinate)/Myvacet-5-07™ (distilled acetylated monoglycerides) emulsions at different concentrations to study their effects on moisture loss and internal gas concentration of zucchini. Zucchini was coated by dipping and by brushing the coating onto the fruit. Both coating applications coated the zucchini completely and uniformly. Researchers determined that dipping was 20% more effective ($p < 0.05$) in reducing water loss than brushing application. They found that the WVR decreased as total solid content of coatings increased. None of the coatings significantly affected internal gas concentration or color of the zucchini.

Park et al. (1994c) reported the effects of an edible corn zein coating on the storage life and quality of tomatoes. Gas transmission rates and thickness of the coating were evaluated. Weight loss, internal gas composition, alcohol content, and color change of the tomatoes were determined. Changes in color, weight, and firmness, as well as sensory quality of coated tomatoes were compared to those of uncoated tomatoes. The lack of ethylene production in coated tomatoes indicated that the coatings slowed ripening and improved storage life. Corn zein coatings delayed ripening when applied at the pink stage and reduced weight loss and firmness of coated tomatoes compared to the uncoated tomatoes. Trained sensory panel results showed that red color development was delayed and softening was accelerated with coated tomatoes. The thinner coatings showed delayed ripening without adverse effects, whereas the thickest coating delayed color development, but had the greatest weight loss and fermentation due to anaerobic

fermentation. Coating thickness directly effected the results of the other data in this study.

Lerdthanangkul and Krochta (1996) studied the effects of milk protein-based edible coatings on green bell peppers. They used coatings made with mineral oil, cellulose, whey protein isolate, sodium caseinate, and sodium caseinate/beeswax emulsion. Glycerol was added only to the milk protein coatings as a plasticizer. They measured changes in respiration, internal gases, color, firmness, and water loss during a 20-day storage period at 10° C and 80 to 85 % RH. The coatings applied to the green bell peppers had no significant effect on the respiration of the green bell peppers. The addition of beeswax to sodium caseinate decreased respiration compared to the sodium caseinate coatings. Cellulose-based and sodium caseinate coatings appeared to be the most effective gas barriers, but they were not effective at high relative humidity. Mineral oil-based coating significantly reduced moisture loss.

During the 1990s, the potential demand for prepackaged minimally processed sliced apples prompted researchers to study the ability of edible coatings to increase their shelf life. Krochta et al. (1990a) coated peeled and sliced apple pieces with an emulsion of casein and acetylated monoglyceride to determine the moisture barrier properties of the coatings. Over a 3-day storage period, the moisture content decreased 8% at room temperature and 0% RH, which was 28% less loss compared to uncoated treatments. Adjusting the caseins pH to its isoelectric point decreased moisture loss by as much as 50% to 70%, depending on coating composition and thickness.

In 1994, Wong et al. coated cut apple pieces with 4 bilayer coatings containing acetylated monoglyceride (AMG) and Avicel, alginate, carrageenan or pectin and

evaluated their gas barrier properties. The apple pieces were dipped in a buffer solution composed of ascorbic acid, citric acid, NaCl, and CaCl₂ before being dipped twice in 0.5% solutions of Avicel™ (microcrystalline cellulose), alginate, carrageenan, or pectin, and then AMG. Results showed a 50% to 70 % reduction in the rate of CO₂ production and a 90% decrease in C₂H₄ at approximately 23°C. They attributed the results to the barrier properties of the acetylated monoglyceride layer and secondarily to the effects of the buffer. The internal O₂ concentration was reduced 50% to 75 %. These results suggested that the change in diffusion rate caused by the coating reduced gas exchange in the coated fruit. All four coatings had a WVR between 38 and 46 sec/cm, which was high compared to the control, which had a WVR of 3.23 sec/cm.

Brancoli et al. (1997) studied the effects of an edible polysaccharide coating containing maltodextrine, methylcellulose, glycerol, ascorbic acid, potassium sorbate, and calcium chloride, as well as storage temperature, on the browning of sliced apples over time. Evaluations were made using a colorimeter and a trained sensory panel. Colorimeter L-value and the computed whitish index data had a correlation coefficient of 0.87 with the sensory data. The polysaccharide coatings significantly inhibited enzymatic browning for 12 days at refrigeration and room temperatures. Refrigeration temperatures also inhibited browning of uncoated samples, but not as effectively as the coating.

1.5. Structural composition and functional properties of milk proteins

Chen (1995) reported that milk proteins contribute little or no flavor or taste to coatings, which makes them attractive for food applications. Researchers reported that

milk proteins are water-soluble and have the ability to act as emulsifiers, which are important functional properties for the formation of edible coatings (McHugh and Krochta, 1994; Chen, 1995). Milk proteins also have potential as microencapsulation agents (Rosenburg and Lee, 1993). Additives such as vitamins, minerals, and colorants can be incorporated into coatings made with milk proteins. Complex intermolecular bindings of milk proteins make coatings good barriers to both oxygen and carbon dioxide, especially at low humidities. Milk proteins have also been effective barriers to lipid oxidation. However, because milk proteins were reported to be hydrophilic in nature, their subsequent coatings were poor water-vapor barriers (McHugh and Krochta, 1994; Chen, 1995). The addition of a lipid to a milk protein-based coating solution may improve the water vapor barrier properties of such coatings.

1.5.1 Characteristics and functionality of caseins

Researchers reported that milk as a complex mixture of lipids, proteins, carbohydrates, vitamins, and minerals. Proteins have been found to make up approximately 3.6 % of the total composition, of which 80% were caseins (Swaigood, 1985; McHugh and Krochta, 1994). The five fractions that comprise caseins were reported to be α_{s1} -casein (34%), α_{s2} -casein (8%), β -casein (25%), κ -casein (9%), and γ -casein (4%) (Swaigood, 1985). Caseins have contained large amounts of phosphoserine residues and a moderately large amount of proline, which has contributed to their hydrophilic character. The β -caseins, which were reported to be the most hydrophobic fraction, have one phosphoserine cluster in the N-terminal, and a large C-terminal, which contributes its hydrophobic properties. Caseins generally have had a secondary and

tertiary random coil structure; however, the proline content in the α_s -caseins has allowed for α -helix, β -sheets, and β -turn structure as well (Brunner, 1977; Swaisgood, 1985).

Both α_{s1} -casein and α_{s2} -casein have been reported to have molecular weights of about 23,000 daltons, were similar in structure and amino acid content, and possessed phosphoserine groups that readily bind to calcium (McHugh and Krochta, 1994). β -caseins were reported to have a molecular weight of 23,900 daltons and were the most hydrophobic of the milk protein fractions. κ -caseins weigh 19,000 daltons and do not actively bind calcium. The N-terminal (residues 1 to 105) was reported to be hydrophobic, whereas the C-terminal (residues 106 to 169) was hydrophilic. Swaisgood (1985) reported that rennet can cleave κ -caseins between residues 105 and 106, leaving the hydrophobic para- κ -casein while the glycomacro-peptide portion is lost in the serum.

When κ -casein associates with α_{s1} -casein and β -casein in the presence of calcium, 80 to 90% of the caseins in milk form thermodynamically stable micelles, which vary in size from 10 to 400 nm or more in diameter. Calcium has reduced electrostatic repulsion inside the micelle, which can lead to hydrophobic interactions (Brunner, 1977; Kinsella, 1984; McHugh and Krochta, 1994). Submicelles rich in κ -casein have been located on the surface of the micelles, and submicelles rich in α - and β -caseins have been found inside. Electrostatic repulsion is reduced and hydrophobic interactions have been facilitated by calcium, which is found inside the micelle (McHugh and Krochta, 1994). The solubility of casein in the micelle has depended on temperature and pH. As temperature decreases, solubility increases because the hydrophobic interactions weaken, allowing them to dissociate from the micelle and solubilize.

Caseins are reported to be amphiphilic in nature. This property has made caseins able to form stable casein-lipid emulsions. The random coil nature of caseins and the intermolecular hydrogen, electrostatic, and hydrophobic bonds they form in solution has made them good candidates for coating formation (McHugh and Krochta, 1994). Casein also could provide structural cohesion, bind the coating to a wet surface, and reduce the waxy appearance of the coating (Krochta et al., 1990b). Surfactant capabilities have enabled caseins to form multicomponent emulsion coatings with better water barrier and mechanical properties (Avena-Bustillos and Krochta, 1993; Krochta et al., 1990b; McHugh and Krochta, 1994). Caseins would also contribute excellent nutritional value to a coated food product (Eigel et al., 1984).

1.5.2 Characteristics and functionality of whey proteins

Whey proteins, which have been reported to account for 20% of total milk proteins are located in the serum portion of milk and are by-products of cheese manufacture. Researchers report that the five fractions that make up whey proteins were β -lactoglobulin (9%), α -lactalbumin (4%), proteose-peptones (4%), bovine serum albumin (BSA) (1%), and immunoglobulins (2%) (McHugh and Krochta, 1994; Swaisgood, 1985). The largest fraction, β -lactoglobulin, makes up 62% of the total whey proteins. Polymorphs A and B have been reported to have similar molecular weights of 18,362 and 18,276 daltons, respectively (McHugh and Krochta, 1994). β -lactoglobulin was reported to be globular in structure, with sulfhydryl and hydrophobic groups inside. β -lactoglobulin has been reported to denature at temperatures above 65 °C exposing the sulfhydryl group and reactive hydrophobic groups (Brunner, 1977), and thereby causing

polymerization (Shimada and Cheftel, 1989). Kinsella and Whitehead (1989) reported that the α -lactalbumin fraction made up 25% of the whey proteins; it has a molecular weight of 14,000 daltons, was globular in structure, possesses four disulfide bonds, and actively binds calcium, which may aid in stabilization of α -lactalbumin. Bovine serum albumin was reported to have a molecular weight of 66,000 daltons, contains 17 disulfide bonds, and was globular in structure. Immunoglobulins and proteose-peptones account for a minute fraction of the whey proteins. The hydrophilic nature of proteose-peptones and the thermal instability of immunoglobulins might adversely affect protein functionality (Kinsella and Whitehead, 1989).

The composition and functional properties of whey proteins have been variable because each fraction has distinctive properties. The solubility and surface activity of whey proteins contribute to the stability of emulsions and foams (Morr and Ha, 1993; McHugh and Krochta, 1994). Thermal denaturation and polymerization processes of whey proteins supported their ability to form water-soluble edible coatings due to the covalent disulfide bonds formed (McHugh and Krochta, 1994; Chen, 1995). The protein-carbohydrate interaction and ligand binding of whey proteins supported their ability to interfere with enzymatic and non-enzymatic browning (Morr and Ha, 1993). Whey protein-based coatings can be excellent oxygen barriers because of the highly polar nature of whey protein and the tightly packed, ordered hydrogen bonded network structure and low solubility. The disulfide bonds formed in whey protein coatings make them suitable for packaging materials (McHugh and Krochta, 1994; Chen, 1995).

1.6. Physiology of apples

1.6.1. Climacteric, respiration, and ripening of apples

The ripening of fruits can be defined as a sequence of changes in color, flavor, and texture that leads to a state at which the fruit is acceptable to eat (Blanpied et al., 1984). Fresh fruits such as apples are living tissues that change continuously after harvest and throughout storage. Askew et al. (1959) reported that apples generally have a moisture content of 85% at the time of maturity. Apples contained 1.24% (fruit weight) glucose and 3.88% fructose (reducing sugars), and 3.8% sucrose at the time of maturity (Askew et al., 1959). The acid content of apples varied among cultivars and can be as low as 7.5 or as high as 13 m-equiv./100 g fruit weight tissue (Johnston et al., 1968).

Climacteric fruits, such as apples, were defined as fruits that produce carbon dioxide and ethylene at suddenly increased rates. This sudden increase in the rate of ethylene production signifies the onset of ripening and is called the climacteric rise (Yahia, 1994). Ethylene production in climacteric fruits has been reported to be autocatalytic in nature (Herner and Sink, 1973; Brackmann and Streif, 1994). The biosynthetic pathway of ethylene production has been determined to be the conversion of S-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC), which is then synthesized to ethylene (Adams and Yang, 1979a,b). The rate-limiting step was in the formation of ACC, which is catalyzed by ACC synthase. Ethylene forming enzyme (EFE) catalyzes the formation of ethylene from ACC and was reported as the last known step in the conversion of ACC to ethylene (Boller et al., 1979; Yu et al., 1979; Yang and Hoffman, 1984). Researchers reported that preclimacteric apples do not have the ability to convert SAM to ethylene because low levels of ACC are present. As the

fruit matures, ACC levels increased with the increase in ethylene production (Hoffman and Yang, 1980; Yang, 1980; Lau et al., 1984). When apples entered the senescence phase, EFE became impaired, ethylene production decreased, and ACC accumulation increased.

Ethylene formation has been reported to initiate the action of enzymes responsible for biochemical processes such as the softening of cell walls, loss of chlorophyll, decrease in acidity, loss of starch, and increase in flavor (Hulme and Rhodes, 1971; Kader, 1985). Most flavor related volatiles are produced after the climacteric rise and reach a maximum in the postclimacteric phase. During ripening, acidity decreased while sweetness and aroma increased (Yahia, 1994). De Pooter et al. (1987) reported that aldehydes are the dominant volatiles detected in intact preclimacteric apples. Mature fruit primarily produced esters and aliphatic alcohols (Flath et al., 1967). Chlorophyll content decreased with climacteric rise (Blanke, 1995).

Studies have shown that pre-climacteric apples can be held without ripening changes when stored under hypobaric (low oxygen, and very low ethylene environment) conditions by uncoupling ethylene production from ethylene action (Bangerth, 1975). The initiation of ethylene climacteric has been regarded as the best measure of pre-ripening physiological development. Deterioration of apples has been directly related to their respiration rate. Tissue injury of apples from minimal processing has triggered a defense response that has rapidly increased production of ethylene, respiration, and formation of secondary metabolites in the apple (Boller and Kende, 1980; Hoffman and Yang, 1982; Rolle and Chism, 1987; Brecht, 1995), which has then increased the rate of senescence (Yahia, 1994; Brecht, 1995). Extended cool storage has resulted in a dry,

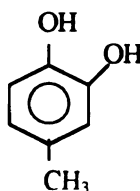
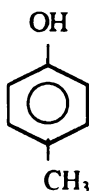
soft, mealy texture of the flesh (Lau et al., 1984; Harker and Hallett, 1992). Minimal processing has caused the breakdown of the cellular membrane and cellular compartmentalization, which has allowed for the mixing of enzymes and substrates leading to undesirable enzyme reactions, such as polyphenol oxidase and phenolic compounds producing melanin to cause browning. Lipase would react with membrane lipids, producing hydroperoxides and fatty acid radicals, leakage of ions and other cellular components, the release of cell wall degrading enzymes, and loss of moisture. Injury also has allowed for growth of microorganisms (Brackett, 1987; Brecht, 1995).

1.6.2 Enzymatic browning of apples

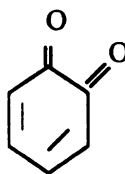
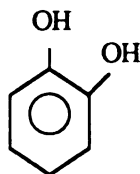
1.6.2.1 PPO and its role. Polyphenol oxidase (PPO) (1,2-benzenediol: oxygen oxidoreductase; EC 1.10.3.1) has been reported to be an oxidoreductase. It is an enzyme that has been known to oxidize diphenols in the presence of oxygen (Vamos-Vigyazo, 1981). PPO has been found in almost all plants in varying concentrations. PPO has contributed the desirable caramel-like color of grapes, prune plums, figs, dates, and tea leaves which contain high concentrations of PPO. The enzyme has been found in moderate amounts in peaches, apples, bananas, potatoes, and lettuce, in which it contributed to undesirable browning reaction (Mayer, 1987; Whitaker, 1996). PPO content, locale and activity has been known to vary among species and cultivar. Researchers reported that PPO activity also depends on species, age, and maturity of the produce (Tolbert, 1973; Siddiq et al., 1994).

Researchers reported that PPO is compartmentalized in the chloroplasts or mitochondria of the cell, and under normal conditions it does not react. Browning has

occurred when PPO, the substrate, and oxygen come in contact due to cell damage caused by bruising, cutting, slicing, or peeling. The PPO activity has been reported to catalyze two different reactions, both requiring oxygen, which indirectly affects enzymatic browning in fruit (Vamos-Vigyazo, 1981; Sapers, 1993; Whitaker, 1996). The first reaction was a hydroxylation of monophenols to o-dihydroxy compounds (Reaction 1). This has been generally known as cresolase activity.



The second reaction is the oxidation of o-dihydroxy phenols to o-quinones. This is generally known as catecholase activity (Reaction 2).



The 4-methyl-o-benzoquinone formed in reaction 2 has been reported to be unstable and undergoes further non-enzyme-catalyzed oxidation by O₂ and polymerization to form melanins (McEvily et al., 1992; Whitaker, 1996).

PPO-catalyzed enzymatic browning has been a major concern in the processed food industry. PPO-catalyzed enzymatic browning has adversely affected the sensory properties and lowers nutritive value of fresh, canned, and frozen plant foods, which can affect their marketability (Vamos-Vigyazo, 1981). Melanins have been reported to be responsible for the brown discoloration in apples (Whitaker, 1996). PPO is found in all parts of the apple. However, it has not been found in clarified apple juice because it remains in the pulp (Vamos-Vigyazo, 1981). Researchers found that the enzymatic browning of apples was correlated with PPO content (Siegelmann, 1955; Weurman and Swain, 1955; Ingle and Hyde, 1968), and a relationship between PPO activity and browning rate (Vamos-Vigyazo et al., 1976). Weurman and Swain observed a decrease in total PPO content during fruit growth, and that a steady level was reached at maturity.

1.6.2.2 Prevention of the browning reaction. Browning can be prevented in fruit by decreasing the oxygen in the system, which is naturally done by the cuticle or peel of the fruit (Whitaker, 1996). If the peel has been removed from the fruit by cutting, peeling, or slicing, placing a barrier to oxygen can eliminate oxygen. One way decreased oxygen has been achieved was with controlled or modified atmospheres that reduce O₂ and/or increase CO₂ inside the package (Rolle and Chism, 1987).

The use of inhibitors such as sulfites that bind to the enzyme and reduce the Cu²⁺ cofactor of the initial product before it completes the reaction and forms melanin, has

been most effective (Sapers, 1993). However, the use of sulfites have been restricted by the FDA due to high occurrence of severe allergic reactions. The immersion of minimally processed fruits and vegetables in acids such as ascorbic acid, cinnamic, and benzoic acid that reduce quinones back to phenolic compounds before the melanins are formed may also be effective (Vamos-Vigyazo, 1981; Sapers et al., 1989). Modification of the substrates by enzymatic methylation may be effective in juices (Finkle and Nelson, 1963). Researchers have found that heat inactivates the PPO enzyme and stops the enzymatic browning to differing degrees in pears (Siddiq et al., 1994), grapes (Siddiq et al., 1992) and potatoes (Batistuti and Lourenco, 1985).

MATERIALS AND METHODS

2.1. Edible coating components and formulation

Whey protein isolate (ALACEN 891) and lactic acid casein (ALACID 710, 30 mesh) samples were obtained from New Zealand Milk Products, Inc., Santa Rosa, CA. Table 1 exhibits the compositional analyses of the whey protein isolate and lactic acid casein as provided by New Zealand Milk Products, Inc. Protein contents were confirmed using AOAC method 981.10 (AOAC, 1990). D-Sorbitol, purchased from Sigma Chemical Co. (St. Louis, MO), was used as a plasticizer. NaOH was obtained from Mallinckrodt Specialty Chemicals Co. (Paris, KY). Unsalted butter was obtained from Land O'Lakes, Inc. (Arden Hills, MN), and carnauba wax USP/NF was obtained from Strahl & Pitsch Inc. (West Babylon, NY).

2.1.1 Milk protein-based edible coating process

Figure 1 displays a schematic diagram of the edible coating process. Edible coating solutions were prepared by mixing lactic acid casein (CAS) or whey protein isolate (WPI) (7.5% w/v) with sorbitol (4, 2.5, 1% w/v), 1M NaOH (to adjust pH), and distilled H₂O to a final volume of 100 mL. The pH of the CAS and WPI-based coating solutions were adjusted to 10 and 8, respectively. Preliminary studies determined that these pH values were optimum for CAS and WPI solubility. Coating solutions were then heated, while being stirred (Model 4820-4 "Magna-4" magnetic stirrer and hot plate, Cole-Parmer, Chicago, IL), to a final temperature of 70 ± 2°C for 45 min for CAS-based solutions, and 90 ± 2°C for 30 min for WPI-based solutions. The pH of each

EDIBLE COATING PROCESS FOR APPLE HALVES

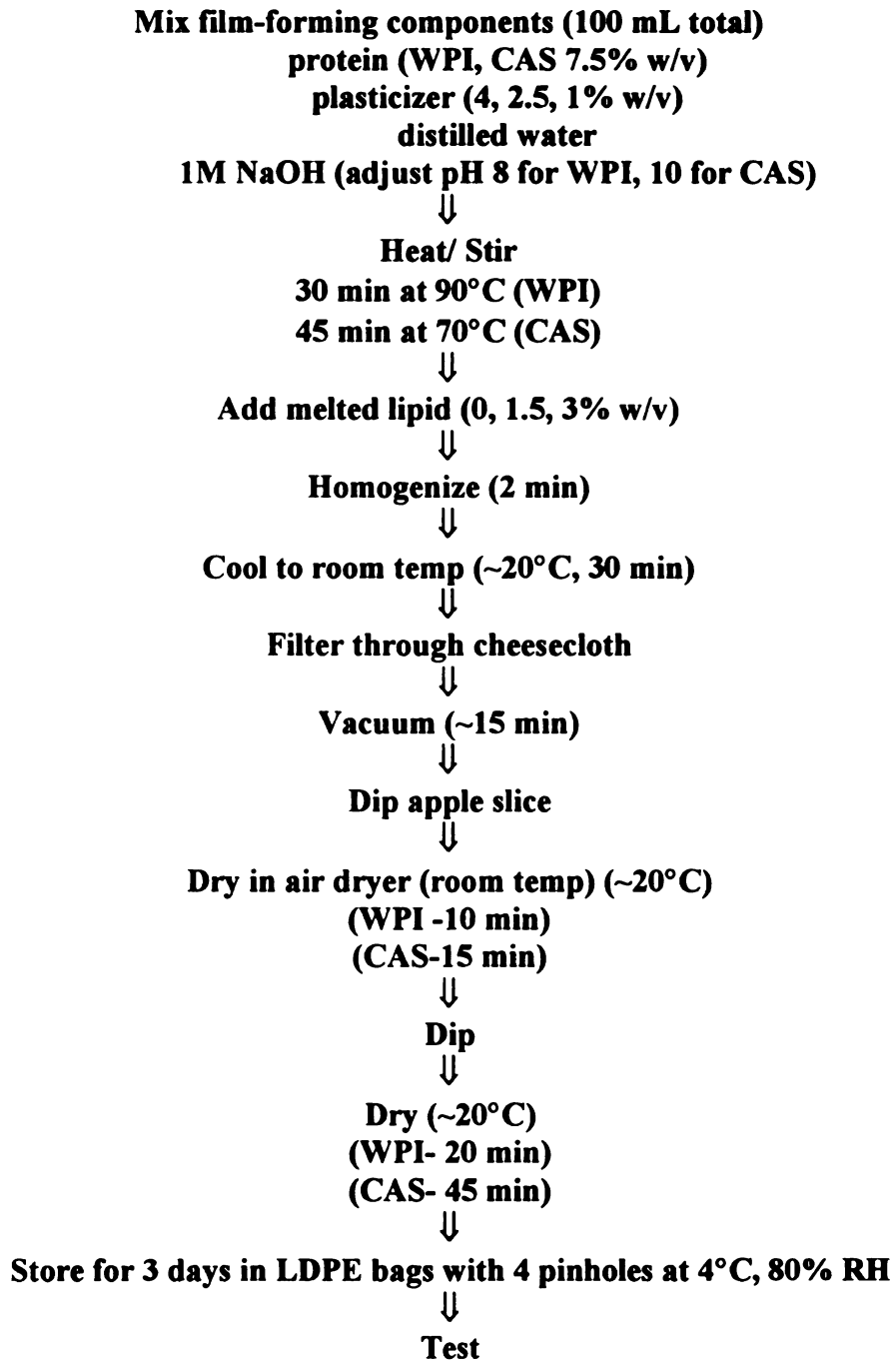


Figure 1. Schematic diagram of the milk protein-based edible coating process used in this study. WPI=wey protein isolate, CAS=lactic acid casein.

Table 1. Whey protein isolate and lactic acid casein composition^a.

Percent (%)	WPI ^b (Alacen 891)	CAS ^c (Alacid 710)
Protein (N x 6.38)	90.0	87.3
Ash %	2.0	1.8
Moisture %	3.8	9.6
Fat %	<1.0	1.2
Lactose %	4.5	0.1
pH	6.5	4.6

^a Values based on specifications provided by New Zealand Milk Products (North America) Inc.

^b WPI=whey protein isolate.

^c CAS=lactic acid casein.

Table 2. Compositions of milk protein-based edible coatings.

Treatments ^a	Protein (g) ^b (w/v)	Sorbitol (g) (w/v)	Lipid (g) (w/v)
1: (7.5:4:0)	7.5	4.0	0.0
2: BF (7.5:2.5:1.5)	7.5	2.5	BF=1.5
3: BF (7.5:1:3)	7.5	1.0	BF=3.0
4: CW (7.5:2.5:1.5)	7.5	2.5	CW=1.5
5: CW (7.5:1:3)	7.5	1.0	CW=3.0

^a BF = butter fat; CW = carnauba wax. (Protein:Plasticizer:Lipid)

^b Each treatment includes one solution made with whey protein isolate and the other made with lactic acid casein as the protein, providing total of 10 treatments.

solution was measured using a Corning pH Meter 240 (Corning, NY). Butter fat (BF) or carnauba wax (CW) (0, 1.5, 3% w/v) was melted and added to the heated solutions. Solutions were then homogenized for 2 min using a Polytron PT 10/35 homogenizer with a PTA 20 TS generator (homogenizing head) and a PCU 11 power control unit at setting 6 (settings range from 1 to 10) (Brinkman Instruments, Switzerland). Edible coating solutions were then cooled to room temperature ($\sim 20^{\circ}\text{C}$) and filtered once through one layer of fine mesh cheesecloth. Edible coating solutions were de-gassed using a hydrometric vacuum system and were transferred to Rubbermaid™ containers and sealed with lids. The covered solutions were set aside for approximately 2 to 3 hours before they were used to coat apple halves. Known volumes of each coating solution were weighed, and density was calculated. Table 2 shows the composition of WPI and CAS-based edible coatings. All percentages reported in the results and discussion are on a weight-per-volume basis unless otherwise noted. The coatings containing BF were not adjusted for the addition of moisture to the coating solution from the butter.

2.2. Edible coating application

2.2.1 Coating of apple halves with milk protein-based edible coatings

Granny Smith and Ida Red apples, common types of apples consumed primarily as a whole fresh fruit, were obtained from a local retail food chain. Each apple was halved (at $\sim 20^{\circ}\text{C}$) approximately 0.5 inches from the center of the core with a serrated kitchen steak knife. The surface area of the halves averaged 44 cm^2 for the Granny Smith and 34.5 cm^2 for the Ida Red. Apple halves were weighed immediately after cutting to

obtain initial weights. Average weights were 40 g and 50 g for Ida Red and Granny Smith, respectively.

The apple halves were then dipped into the edible coating solution ($\sim 20^{\circ}\text{C}$). Excess coating was drained by gently shaking the apple halves. Coated apple halves were weighed to determine wet coating weight. The coated apple halves were dried ($\sim 20^{\circ}\text{C}$) in an Easy Breeze™ Gel Dryer (Hoefer Scientific Instruments, San Francisco, CA) for 10 min (WPI-based) or 15 min (CAS-based). The apples were re-weighed to determine weight loss, then dipped and drained again. The re-coated apples were then dried at room temperature until the coating appeared to be completely dry (20 min for WPI-based, 45 min for CAS-based). All samples were weighed to determine total weight loss due to drying. Uncoated apple halves were used as controls.

Data for day 0 were obtained immediately after the coatings were dry. All samples were placed in low-density polyethylene (LDPE) bags (thickness = 1.75 mil) with 4 small pinholes to prevent anaerobic conditions. The bags were stored in a cooler at $5 \pm 1^{\circ}\text{C}$, $80 \pm 8\%$ RH for 3 days. Percent RH inside LDPE bags was monitored each day.

2.2.2 Determining the weight of milk protein-based coatings applied to apple halves

The weight of dry coating applied could not simply be determined by subtracting the weight of the coated apple half from the weight of the uncoated half because the apple halves themselves may have lost moisture during the drying period. Therefore, the weight of coating solution applied was determined and then dried on an inert surface to determine dry coating weight on the apple half. The weight of the coating on the apple

half was determined by weighing the apple half: 1) before the first edible coating application, 2) after the wet edible coating was applied, 3) when the first coat was dry, 4) after the second wet coat was applied, and 5) when the edible coating was completely dried. The amount of wet edible coating was calculated by subtracting the weight of the apple half after the first dipping from the initial uncoated weight. The weight of the apple half after the second dipping was then subtracted from their weight with a dried first coat. These two values were then added to determine the weight of the wet coating per apple half. To determine the weight of the dry coating, the amount of edible coating solution calculated above was pipetted into a watch glass and left to dry at (20°C, 40% RH, ~ 1 hour). The weight of the dried coating was then recorded as the approximate weight of coating used on each apple half. This weight was subtracted from the total weight (of the coated apple half) to determine the weight loss of the apple half alone.

2.3. Determination of physical properties

2.3.1 Viscosity of milk protein-based edible coating solutions

Tamura et al. (1993) reported that an understanding of the rheological properties of food is necessary in the development and optimization of new products, process methodology, and final product quality. Viscosity has affected heat transfer characteristics and flow behavior, and has been a key quality indicator (Rao and Ananteswaran, 1982; Tamura et al., 1993). Rotational instruments have been capable of measuring normal stress differences and dynamic properties of solid or semi-solid foods by means of compression, tension, or torsion, which can best be achieved between two plates or in a mixing function (Vercruysse and Steffe, 1989; Tamura et al., 1993; Steffe,

1996). Rotational instruments have been suitable for Newtonian and non-Newtonian fluids where time dependant behavior is generally investigated (Tamura et al., 1993). Cone-and-plate systems are best used with homogeneous products.

The viscosity of each coating solution was measured using the HAAKE Rheostress RS100 with cone-and-plate geometry (Paramus, NJ). Coating solution (4 mL) was pipetted onto the 25°C flat 60 mm plate. The temperature of the solution was kept constant using the Haake F3 waterbath (Paramus, NJ) system. The cone (C60 mm/4° angle) was lowered to within 0.131 mm of the plate to collect the data. Data were collected for shear rates ($\dot{\gamma}$) of 10 s⁻¹ to 100 s⁻¹. Apparent viscosity was calculated and graphed over time using the RS100 version 1.4 software (Paramus, NJ). If the graph was linear, the coating solutions were thought to be Newtonian. If the solutions loose viscosity or thicken with shear, they would be classified as shear thinning or shear thickening fluids, respectively. The shear rate range used in this study was obtained from a table listing suggested ranges of shear rates to evaluate fluids (Steffe, 1996).

2.3.2 Thickness of the dried milk protein-based edible coatings on the apple halves

The thickness of each edible coating applied to the apple halves was determined using a modified procedure of Park et al. (1994a). A Bausch & Lomb DynaZoom microscope (Rochester, NY) with a flat-field inclined monocular was used at a magnification of 100x. A red water-based food dye (Durkee-French Foods, Wayne, NJ) was incorporated into the coating solutions. The apple halves were coated as previously described in section 2.2. The coated apple halves were stored at 5 ± 1°C, 80 ± 8% RH for 24 hrs. Cross-sections were cut lengthwise and widthwise, as illustrated in Figure 2, and

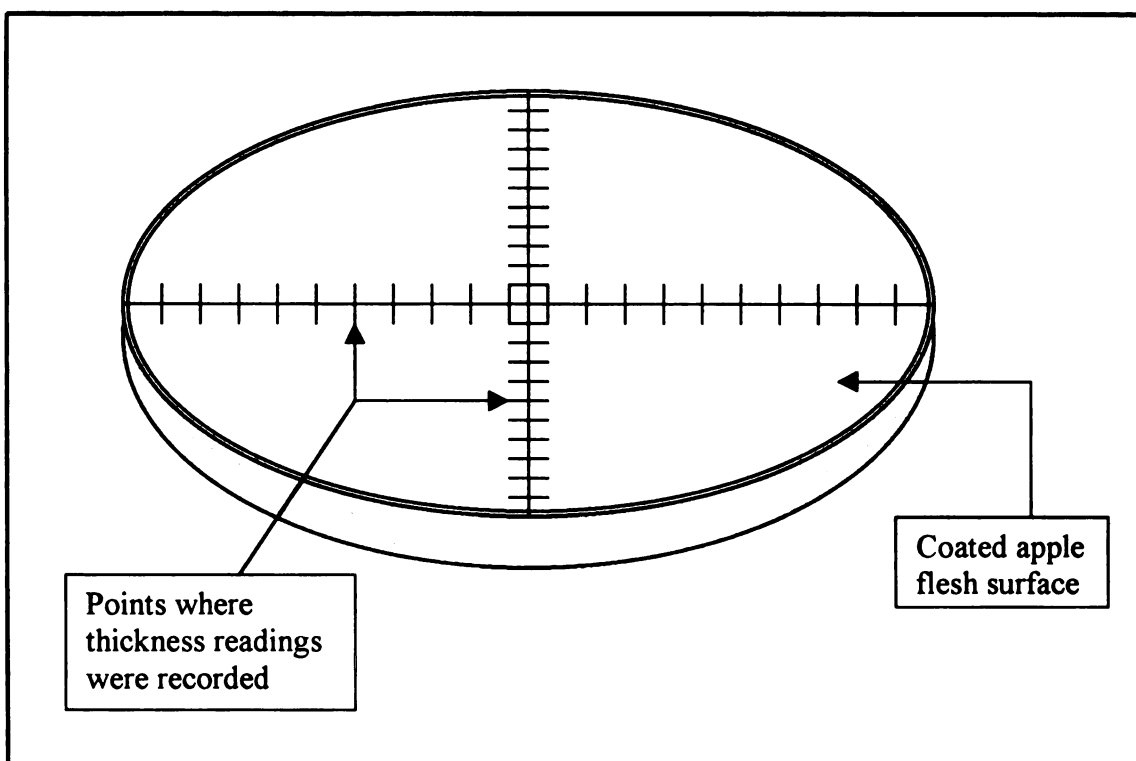


Figure 2. Schematic diagram for the determination of the thickness of the dried milk protein-based edible coatings applied to apple halves. Hash marks vertically and horizontally denote where thickness was observed and recorded.

placed on a glass microscope slide for viewing. Thickness was measured using a micron scale. Twenty readings were obtained from each cross-section and reported as average thickness.

2.3.3 Surface continuity of the dried milk protein-based edible coatings on the apple halves and on a plastic surface

A Nikon SMZ-U stereomicroscope with a Sony Hyperhead HAD color video camera and Fostec EKE Ringlight attachment (Mager Scientific, Dexter, MI) powered by a Pentium 233 Microsolutions computer was used to observe the surface continuity of the coating on the apple flesh and on a plastic petri dish. To evaluate the continuity of the coating on the apple flesh, cross-sections were taken through the centers of the coated apple halves. The cross-section samples were placed on a plastic petri dish and observed at 1.5x magnification. To observe the general continuity of the coating dried on an inert surface, coating solutions were dyed with a red food dye (Durkee-French Foods) and allowed to stand 1 hour. Samples (2 mL) were spread on a plastic petri dish in a 36 cm² area and allowed to dry. Samples were observed under 5.5x magnification. Figure 3 displays the system design. Images of the cross-sections and dry coating solutions were taken using Snappy Digitizing software, version 2 (Play Co., Rancho Cordova, CA).

2.4. Chemical properties of Ida Red and Granny Smith apples

2.4.1 PPO extraction from Ida Red and Granny Smith apples

Polyphenol oxidase (PPO) content of each apple cultivar was determined and correlated to the degree of browning. PPO was extracted using a modified method of Siddiq et al.

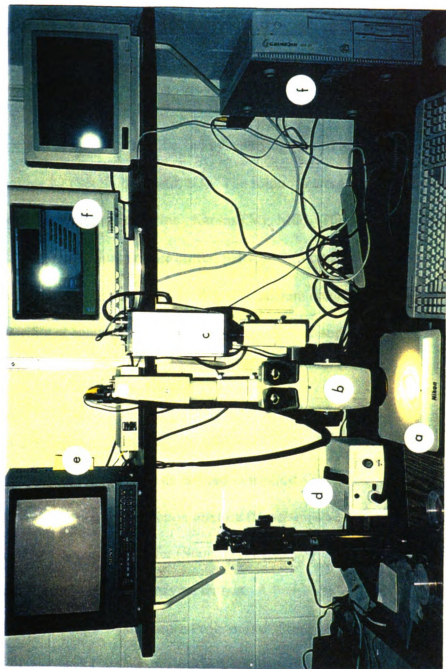


Figure 3. Equipment set-up for determining thickness and continuity of milk protein-based edible coatings. a) Sample platform, b) Nikon SMZ-U Stereomicroscope, c) Sony Hyperhead HAD color Video camera, d) Fostec EKE Ringlight attachment, e) Videocamera monitor, f) Pentium 233 Microsolutions computer and monitor.

(1992). All materials used for extraction were maintained at $5 \pm 1^\circ\text{C}$ to reduce enzyme activity during extraction. A random sample of 50 g of tissue from 5 similar-sized apples was blended in a Waring blender (New Hartford, CT) with a 100 mL solution of 0.1M Tris hydroxymethylaminomethane (Trizma) buffer (Sigma) (pH 9.5, 5°C) for 2 min. This mixture was filtered through 8 layers of cheesecloth. The filtrate was precipitated by slowly adding 100 mL of -20°C acetone (Mallinckrodt, Inc., Paris, KY) while stirring gently. Precipitate was collected by centrifuging at 4°C , $1735 \times g$, for 3 min in a Heraeus Biofuge 22 (South Plainfield, NJ). The solutions were strained through Nitex 3-45/29 mesh nylon cloth (Tetko, Inc., Kansas City, MO). The precipitate was suspended in 50 mL of 0.1M sodium acetate buffer, pH 7, 5°C (Sigma). Pectic substances were precipitated by adding 8.0 mL of 0.05M calcium chloride (Sigma). The solution was centrifuged again at $2711 \times g$ for 10 minutes. The supernatant was used for the enzyme assay.

2.4.2 Enzyme activity assay

Activity of PPO was assayed in triplicate, according to the method of Cash et al. (1976). The standard reaction mixture (Coseteng and Lee, 1987) consisted of 2.6 mL of 0.1M sodium acetate buffer (Sigma) (pH 6.0), 0.3 mL of 0.5M catechol (Sigma), and 0.1 mL of freshly prepared enzyme extract. A Perkin-Elmer UV/MS Spectrometer Lambda 20 with a Peltier Temperature Programmer (Norwalk, CT) equilibrated at 30°C and equipped with UVwinLab version 2 software (Perkin Elmer Corp, 1994-96, Norwalk, CT) was used to determine change in absorbance at 420 nm for 4 min. The increase in absorbance was recorded at 10 sec intervals. One unit of enzyme activity was calculated

from the slope of the curve, which determined $\Delta A_{420\text{nm}}/\text{min}$ due to the oxidation of catechol (i.e., 1 unit = change in absorbance of 0.001/min).

2.4.3 Determination of protein content in apples

Protein content of the apple PPO extract was determined following the methodology adopted from Bradford (1976), using the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA). A shift in the absorption maximum of the protein from 465 to 590 nm, as a result of dye binding, was measured to determine protein content. Aliquots (1 mL) of BSA were prepared and stored in a freezer at -10°C until needed. Protein concentration (mg protein/g apple) of the aliquots prepared as above was determined at the time of use using the Perkin-Elmer UV/MS Spectrometer Lambda 20 equilibrated to 30°C at 280 nm using double-distilled water as a blank. The standard BSA dilution was then calculated by dividing the absorbance by 6.67. Eight dilutions of protein standard were prepared. Amounts containing 0-500 μg of BSA (0.0-0.500 mL) standard solution were used for protein measurement. The absorbance was measured at 590 nm with a V_{max} Kinetic Microplate Reader with SoftMax version 234 software (Molecular Devices Co., Menlo Park, CA). The standard curve prepared with the BSA was used to calculate the protein content of different apple enzyme preparations.

2.5. Barrier properties of milk protein-based edible coatings

2.5.1 Total moisture loss of coated and uncoated apple halves

Total moisture loss of the coated apple halves was measured. Moisture loss data were collected by weighing the coated and uncoated apple halves at time 0, and then once

a day for 3 days. Total moisture loss was determined by calculating the difference in weight between day 3 and day 0.

2.5.2 Moisture-loss rate of coated and uncoated apple halves

Moisture-loss rate was determined according to the method used by Avena-Bustillos et al. (1994a). The time of each data measurement was recorded so that the moisture-loss rate could be calculated. The slope of a graph of weight loss versus time was determined to indicate the moisture-loss rate. Moisture-loss rate was used in the calculation of water vapor resistance.

2.5.3 Water vapor resistance (WVR) of coated and uncoated apple halves

The surface area of each apple was measured to determine the amount of coating applied to each sample and to calculate WVR using a modified Fick's first law equation (Ben-Yehoshua et al. 1985):

$$r = \left[\frac{\left(A_w - \frac{\%RH}{100} \right) P_{wv}}{RT} \right] \left(\frac{A}{J} \right)$$

Where:

r = Water vapor resistance (s/cm)

A_w = Water activity, 0.982 (Guegov, 1980; Chirife and Ferro-Fontan, 1982)

% RH = Average storage room relative humidity

P_{wv} = Saturated water vapor pressure (mm Hg)

R = Universal gas constant (3464.62 mm Hg*cm³/g*°K)

T = Storage room temperature (°K)

A = Surface area of sample (cm²)

J = Weight-loss rate (g/sec)

2.5.4 Oxygen permeability of coated and uncoated apple halves

The internal O₂ content of the coated and uncoated apple halves was evaluated by determining the percent of internal O₂ in the coated apple halves at steady state. The three best WPI and the two best CAS-based coatings, as determined in the color and WVR experiments (described in section 2.5.5 and 2.5.3, respectively), were selected for this study. Coated apple halves were prepared as described in section 2.2 and stored over 2 days in $4 \pm 1^\circ\text{C}$, $95 \pm 2\%$ RH. Steady state was reached at 24 hours, at which time internal O₂ was determined. Data were confirmed by determining the percentage of internal O₂ again at 48 hours. A vacuum system was developed using a desiccator jar with a HYVAC 7 vacuum pump (Central Scientific Co., Cenco Instruments Corp., Chicago, IL) connected through a septum in the lid of the desiccator (Figure 4a). Coated apple halves were placed under an inverted funnel, which was immersed in a de-aerated, saturated calcium chloride solution (Sigma). A silicone self-sealing septum placed at the opening of the stem before completely immersing the funnel into the solution, filling the entire funnel and completely displaced any oxygen from the air. The de-aerated, saturated CaCl₂ solution was used to completely displace oxygen in the system to ensure that the oxygen pulled by the vacuum would only be that from the interior of the apple slice. As the vacuum was applied, the O₂ from the interior of the apple half rose to the stem of the funnel. The vacuum was turned off when the oxygen from the apple filled half of the stem of the funnel. A Monoject 1/2cc insulin gas tight syringe (Sherwood Medical, St. Louis, MO) was inserted into the septum to extract a 10μL sample of air, which was then injected into Paramagnetic Oxygen Analyzer (Servomex Series 1100 with a paramagnetic detection cell, Crowborough, Sussex, England) connected in series. N₂

was the carrier gas (flow rate = 100 mL/min). The percent of interior O₂ in the samples was recorded on a chart recorder (Alltech Associate Industries, Deerfield, IL) (Figure 4b), and compared against a standard sample of air (20.7%) and a known standard with a lower oxygen concentration.

2.5.5 Instrumental determination of browning of coated and uncoated apple halves

Browning of the coated apple halves was evaluated using the HunterLab colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). L-value (black to white) was determined from the color scale after calibrating with a standard white tile (Sapers and Douglas, 1987). A black tile was used as a background. The difference in L-value from day 0 to day 3 was determined and compared to the uncoated control.

2.6 Browning and overall acceptance as determined by a trained sensory panel

Browning and overall acceptance of coated apple halves over time was evaluated using an 11-member trained sensory panel consisting of graduate students and research associates at Michigan State University. The panelists were selected through a screening process that determined the panelist's ability to distinguish between very slight color changes in apple flesh. The selected panelists participated in a short orientation and 3 training sessions. They were trained to recognize color differences of two cultivars of apple slices over time, and to recognize color change extremes amongst each apple cultivar. Panelists practiced using a structured intensity scale for the degree of color change, and overall acceptance of the appearance. They were then provided feedback on

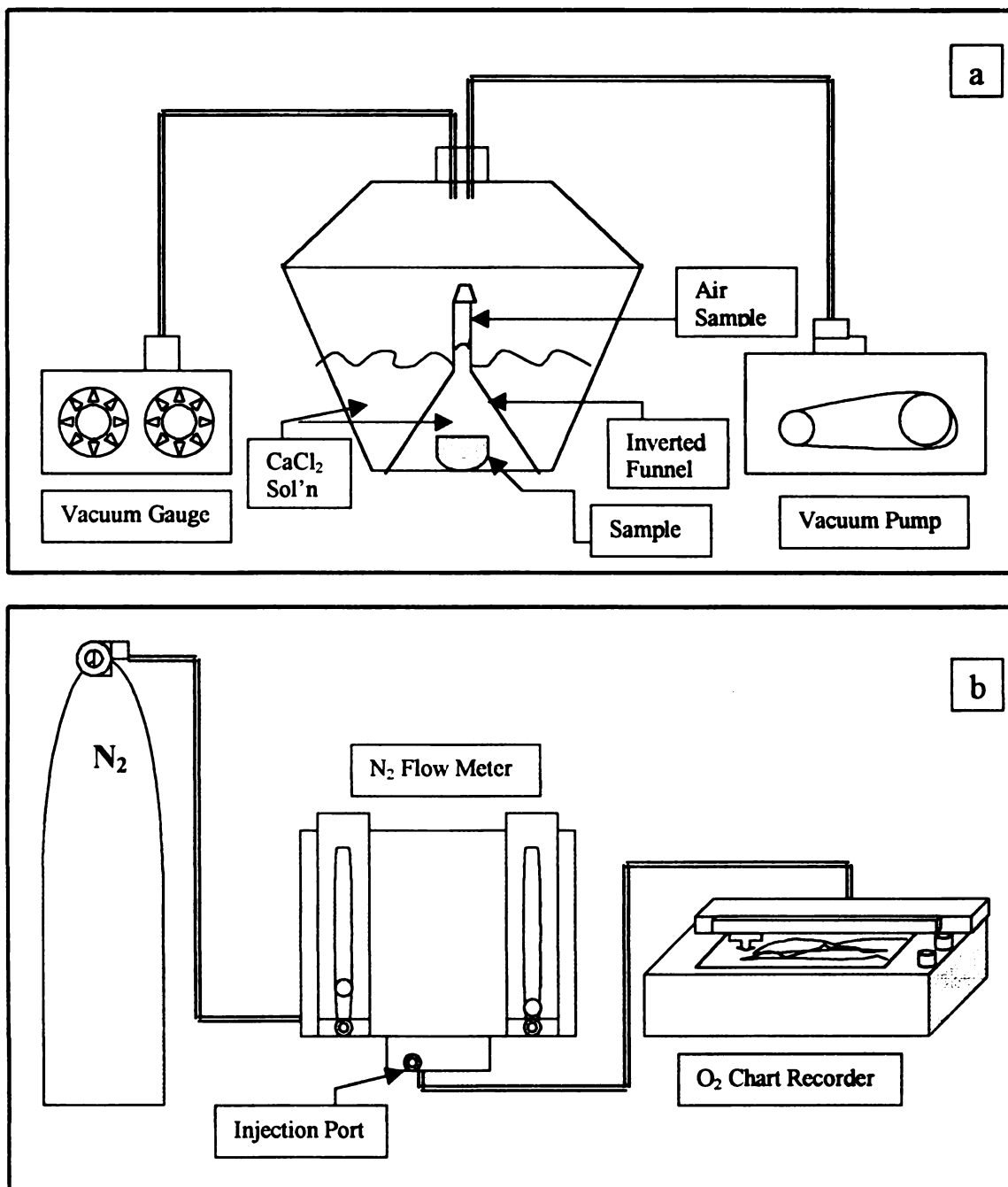


Figure 4. Schematic diagram for the determination of gas permeability.
 a) Oxygen collection set-up b) Oxygen analyzing system

their results. Panel sessions were intended to be held every 48 hrs for 14 days, but were terminated after 6 days due to excessive deterioration of samples. Panelists evaluated up to 6 sets of samples per session. Samples evaluated consisted of 2 WPI-based coated samples and 1 CAS-based coated sample, which were determined to provide the best color protection and WVR as determined in section 2.5. Comparisons were made to uncoated controls. All sessions were held in a climate controlled sensory laboratory equipped with individual testing booths. Three coated samples and the uncoated control, were placed on a styrofoam tray and labeled with randomly selected 3-digit numbers. The panel was instructed to evaluate samples visually for degree of browning and for overall acceptance of appearance. Panelists evaluated the degree of browning using a structured 9-point intensity scale where 1 indicated not brown (fresh cut), and 9 indicated very brown (Appendix 1). Overall acceptance was evaluated using a 5-point hedonic scale where 1 indicated unacceptable, and 5 indicated very acceptable (Appendix 1). Sensory scores were averaged for 11 judges for each of the 3 replicates.

2.7. Shelf life of coated and uncoated apple halves during refrigerated storage

Two WPI and one CAS-based coating that provided the best color protection and WVR as determined in section 2.5 were used in a 14-day storage study. Apple halves were coated as described in section 2.2 and stored at $5 \pm 1^{\circ}\text{C}$, $80 \pm 8\%$ RH for 14 days. Samples were evaluated at day 0 and then at 2-day intervals for a total of 14 days. Samples were compared to uncoated controls for weight loss, WVR, and color change as described in section 2.5. In addition, these coated apple halves were evaluated by the

trained panel for browning during the 14-day storage study as described in section 2.5.6. to validate the results obtained by the colorimeter.

2.8. Statistical analysis

Browning, total moisture-loss, moisture-loss rate, and WVR experiments were replicated 4 times in randomized design experiments. In these experiments, the coated samples were compared to the uncoated controls using the Student-Newman-Keuls method for multiple and pairwise comparisons. Statistical analysis was also performed between protein type applied (WPI -vs- CAS) to the same apple cultivar, and comparisons were performed using the student t-test.

The enzyme activity assay, protein, viscosity, and density determination were replicated 3 times. Comparisons were made between apple cultivar for the enzyme activity assay and protein determination. Comparisons were made between coating samples for the viscosity and density using the Student-Newman-Keuls method for multiple and pairwise comparisons.

Oxygen determination and the storage study were replicated 3 times. Coated samples were compared to the uncoated control using the Student-Newman-Keuls method for multiple and pairwise comparisons.

Additional visual panel statistical analyses were performed using the three-way ANOVA and the Student-Newman-Keuls method for multiple comparisons. Visual panel results were correlated to colorimeter results using regression analysis.

All statistical analyses were made using SigmaStat 2.0 (Jandel, San Rafael, CA).

RESULTS AND DISCUSSION

3.1 Physical properties of milk protein-based edible coatings

Kjeldahl analysis showed that WPI and CAS powders had 89.9% and 85.7% protein, respectively. The protein contents varied by $\leq 3\%$ compared to those values provided by New Zealand Milk Products, Inc. (Table 1).

Most coating solutions had a slight yellow tint and the solutions with lipid appeared cloudy. The CAS-based coating solution without lipid appeared white. The WPI-based coating solution containing no lipid was transparent. CAS-based coating solutions were more viscous than the WPI-based coatings. On the apple flesh, dried WPI and CAS-based coatings were transparent, with a shiny surface. However, the coatings containing BF showed evidence of a white precipitate on the apple flesh. A reaction between the calcium in the coating and the acidic surface of the apple flesh may have caused a salting out of the calcium.

3.1.1 Dried coating weight of milk protein-based edible coatings applied to apple halves

Table 3 shows the weights of the dried WPI and CAS-based coatings applied to apple halves. Dried coating weights within the WPI-based coatings on Granny Smith and Ida Red apple halves were similar. The weights within the CAS-based coatings were similar except the CAS-based coating applied to Ida Red halves with no lipid incorporated which was heaviest ($p < 0.05$) compared to the other CAS-based coatings (Table 3). The WPI and CAS-based coatings composed of 3% BF were similar, however lighter in

Table 3. Weight of dried milk protein-based edible coatings applied to apple halves.

Dry Weight ^{a,b} (mg/cm ²)				
Treatments ^c	Ida Red		Granny Smith	
	WPI	CAS	WPI	CAS
1: (7.5:4:0)	6.38 ± 0.33 ^d	12.17 ± 0.93 ^d	7.15 ± 0.46 ^d	11.81 ± 1.74 ^d
2: BF (7.5:2.5:1.5)	6.46 ± 0.77 ^d	10.06 ± 0.84 ^e	7.20 ± 0.96 ^d	10.05 ± 1.03 ^{de}
3: BF (7.5:1:3)	5.51 ± 1.27 ^d	8.40 ± 0.71 ^e	6.92 ± 0.86 ^d	9.06 ± 0.29 ^e
4: CW (7.5:2.5:1.5)	6.78 ± 0.64 ^d	10.11 ± 1.18 ^e	7.11 ± 0.24 ^d	11.18 ± 0.92 ^{de}
5: CW (7.5:1:3)	7.27 ± 0.45 ^d	8.73 ± 0.94 ^e	7.18 ± 0.21 ^d	9.57 ± 1.03 ^{de}

^a WPI= whey protein isolate; CAS= lactic acid casein.

^b Means ± standard deviation; n=4 for all treatments.

^c BF = butter fat; CW = carnauba wax. (Protein:Plasticizer:Lipid)

^{d-e} columnwise denote significant difference (p<0.05).

Table 4. Viscosity of milk protein-based edible coatings (@ 25°C)

Viscosity (mPa•s) ^{a,b}		
Treatments ^c	WPI ^d	CAS ^e
1: (7.5:4:0)	9.27 ± 2.28	50.70 ± 10.80
2: BF (7.5:2.5:1.5)	8.07 ± 0.98	42.50 ± 6.20
3: BF (7.5:1:3)	7.63 ± 0.50	57.10 ± 11.70
4: CW (7.5:2.5:1.5)	8.03 ± 0.59	63.50 ± 9.24
5: CW (7.5:1:3)	10.00 ± 2.40	76.40 ± 40.40

^a WPI= whey protein isolate; CAS= lactic acid casein.

^b Means ± standard deviations; n=3 for all treatments. No significant differences were observed within protein types.

^c BF= butter fat, CW= carnauba wax. (Protein:Plasticizer:Lipid)

^{d-e} denote significant differences between protein type (p<0.05).

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weight than all other coatings applied to both apple cultivars. The WPI-based coatings containing 3% CW were heaviest of the WPI-based coatings, whereas the CAS-based coatings without lipid were heaviest of the CAS-based coatings. The CAS-based coating with 3% BF applied to Granny Smith apple halves was the lighter ($p < 0.05$) than the CAS-based coating without lipid. Dried WPI-based coating on Ida Red and Granny Smith apple halves was lighter in weight than the CAS-based coatings indicating that more CAS coating was applied to the apple halves which may form a thicker coating on the apple flesh when dried. The CAS-based coatings were expected to be more dense and thicker than the other coatings of the same protein type because they weighed more.

3.1.2 Viscosity of the milk protein-based coating solutions

The CAS-based coating solutions were more ($p < 0.05$) viscous than the WPI-based coating solutions. Viscosities of the coating solutions within the same protein type were similar (Table 4). The WPI and CAS-based coating solutions containing 3% CW had higher viscosities than the others. The viscosity for the 3rd rep data point of the CAS-based coating solution containing 3% CW was twice that of the other two reps, which accounts for the exceptionally high standard deviation for that data set. Typically more viscous coating solutions formed thicker coatings on the apple halves. Coating viscosity may also affect the uniformity of the applied coating on the apple half (Brake and Fennema, 1993). All of the coating solutions in this study exhibited Newtonian behavior. A Newtonian fluid has been reported to be inelastic, and the apparent viscosity, $\eta(\gamma)$, constant and equal to the Newtonian viscosity (Saravacos, 1970; Khalil et al., 1989; Ibarz et al., 1994; Steffe, 1996). The behavior of an inelastic fluid has been identified by a plot

of shear stress versus shear rate in a directly proportional relationship (Ibarz et al., 1992b). Shear stress has been defined as a force per unit area, expressed as Pascal (N/m²). Fluids can be studied by subjecting them to continuous shearing at a constant rate, using a cone-and-plate or parallel-plate rheometer (Ramana and Taylor, 1992; Steffe, 1996). Results of shear stress (σ) versus shear rate (γ) of the coating solutions showed a linear relationship, with $R^2 = .99 \pm .01$ indicating Newtonian behavior. This was inconsistent with that reported by Mate and Krochta (1996), who examined the viscosity of WPI (11% w/w) / glycerol coatings in a 3:2 and 1:1 (w/w) ratio and reported them to exhibit shear thinning behavior. The Newtonian behavior exhibited by the coating solutions in the present study could be attributed to a lower percentage of protein/plasticizer than the coating solutions developed by Mate and Krochta (1996). Coatings with Newtonian behavior would be easier to use in an industrial application because the viscosity would not change with temperature or when stress or strain is applied.

3.1.3 Density of the milk protein-based edible coating solutions

Table 5 shows the densities of the coating solutions developed in this study. WPI-based coating solutions had lower ($p < 0.05$) densities than CAS-based coating solutions. The densities within the WPI-based coating solutions were similar. All CAS-based coating solutions were similar except the coating solution without lipid and the coating solution containing 1.5% CW (1.05mg/mL) which were higher ($p < 0.05$) than the others. The densities of the coating solutions corresponded with the dried weights reported in section 3.1.1 except the WPI-based coating without lipid and the CAS-based coating

Table 5. Density of milk protein-based edible coating solutions (@20°C).

Density (mg/mL) ^{a,b}		
Treatments ^c	WPI ^f	CAS ^g
1: (7.5:4:0)	1.03 ± 0.00 ^d	1.05 ± 0.01 ^d
2: BF (7.5:2.5:1.5)	1.02 ± 0.01 ^d	1.04 ± 0.01 ^e
3: BF (7.5:1:3)	1.01 ± 0.01 ^d	1.03 ± 0.00 ^e
4: CW (7.5:2.5:1.5)	1.03 ± 0.02 ^d	1.05 ± 0.00 ^d
5: CW (7.5:1:3)	1.03 ± 0.01 ^d	1.03 ± 0.01 ^e

^a WPI= whey protein isolate; CAS= lactic acid casein.

^b Means ± standard deviations; n=3 for all treatments.

^c BF= butter fat, CW= carnauba wax. (Protein:Plasticizer:Lipid)

^{d-e} columnwise denote significant differences (p<0.05).

^{f-g} denote significant difference between protein types (p<0.05).

Table 6. Thickness of milk protein-based edible coatings applied to apple halves.

Average Thickness (μm) ^{a,b}				
Treatments ^c	Ida Red		Granny Smith	
	WPI	CAS	WPI	CAS
1: (7.5:4:0)	32.39	39.60	32.98	43.56
2: BF (7.5:2.5:1.5)	31.43	36.08	28.26	41.02
3: BF (7.5:1:3)	25.72	36.45	29.31	36.10
4: CW (7.5:2.5:1.5)	27.40	35.48	26.83	38.24
5: CW (7.5:1:3)	28.25	35.04	27.92	35.64

^a WPI= whey protein isolate; CAS= lactic acid casein.

^b n=40 for all treatments. No statistics were performed.

^c BF= butter fat, CW= carnauba wax. (Protein:Plasticizer:Lipid)

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containing 1.5% CW which had the higher densities, however they did not have the higher dried weight for their protein type. The CAS-based coating solutions had both higher viscosity and densities than the WPI-based coating solutions ($p < 0.05$).

3.1.4 Thickness of dried milk protein-based edible coatings applied to apple halves

Thickness data was not statistically analyzed as only one set of data was collected. Table 6 shows the thickness measurements of milk protein-based coatings applied to apple halves. These values were estimations of the coating thickness as measured through a microscope. Thickness was not uniform on the surfaces of the samples. Also, it was difficult to determine an accurate thickness because some of the coating material (enhanced in the figures using food coloring) migrated into the apple tissue (Figure 5 and 6).

Generally, CAS-based coatings visually appeared thicker than WPI-based coatings applied to the same apple cultivar for any treatment. This was expected in terms of the coating viscosities reported in section 3.1.2. A more viscous solution would leave more coating on the apple half due to less run-off, and would dry as a thicker coating. WPI-based coating with 3% BF and CAS-based coating with 3% CW were the thinner coatings on Ida Red apple halves (25.72 μm and 35.04 μm , respectively). Of the coatings applied to Granny Smith apple halves, the WPI-based coating adding 1.5% CW and the CAS-based coating with 3% CW were thinner than the others (26.83 μm and 35.64 μm , respectively). WPI and CAS-based coatings without lipid were thicker when applied to both apple cultivars (Table 6).

Mate et al. (1996) reported a direct relationship between the final weight of coating applied and its thickness. The coatings applied to apple halves in this study did not show this trend. The average thickness of the WPI/glycerol coating applied to peanuts in the Mate et al. study was 197 to 216 μm when peanuts were dipped 3 times. Mate and Krochta (1996) reported that the average coating thickness of WPI/glycerol coatings applied to peanuts ranged from 111 μm to 144 μm for peanuts dipped twice, which was more than twice as thick as the coatings in this study. However, the protein concentration they used in their coating solutions was higher than the protein concentration in this study. The coatings in this study were thicker than Semperfresh™ coatings applied to whole apples, which ranged from 4.5 μm to 13.2 μm , depending on the concentration of Semperfresh™ used in the coating (the higher the concentration the thicker was the coating) (Park et al., 1994a). The use of polysaccharide instead of protein to form the coating matrix may also have accounted for the difference in coating thickness.

3.1.5 Surface appearance and continuity of milk protein-based edible coatings

WPI and CAS-based coatings on both apple cultivars appeared to have formed a continuous coating on the cut surface of the apple halves, with some migration into the flesh. However, the WPI-based coating applied to Ida Red and Granny Smith apple halves with 3% BF (Fig. 5e and 6e), and the WPI-based coating applied to Granny Smith halves containing 3% CW (Fig. 6i) did not appear to have migrated into the apple flesh. The migration of the coating into the apple flesh made it difficult to determine the accurate thickness of the coatings, contributing to the variability of the thickness measurement.

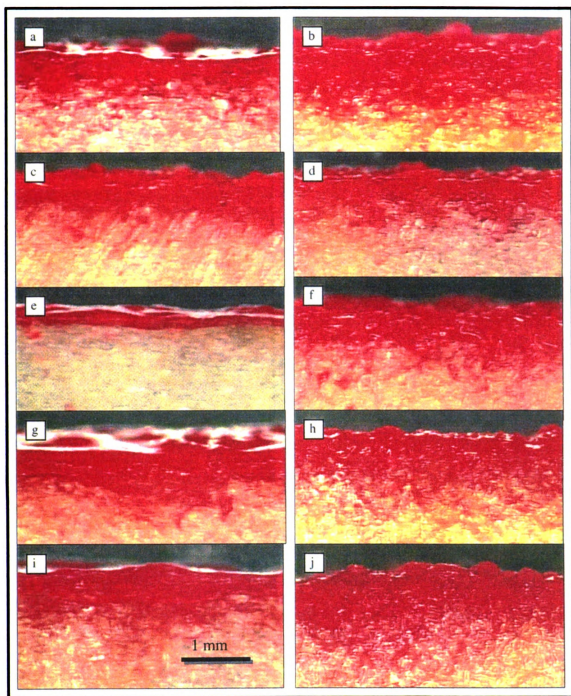


Figure 5. Cross-sections of Ida Red apple halves coated with milk protein-based edible coatings. a) Whey protein isolate (WPI)-no lipid; b) Lactic acid casein (CAS)-no lipid; c) WPI-1.5% butter fat (BF); d) CAS-1.5% BF; e) WPI-3% BF; f) CAS-3% BF; g) WPI-1.5% carnauba wax (CW); h) CAS-1.5% CW; i) WPI-3% CW; j) CAS-3% CW.

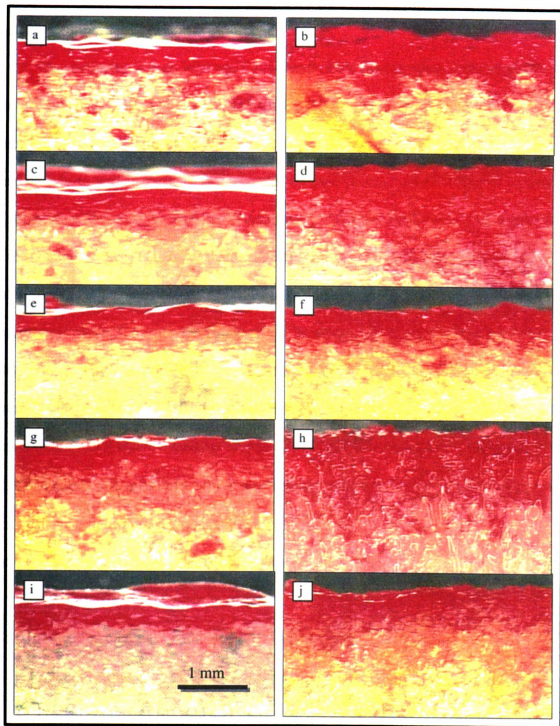


Figure 6. Cross-sections of Granny Smith apple halves coated with milk protein-based edible coatings. a) Whey protein isolate (WPI)-no lipid; b) Lactic acid casein (CAS)-no lipid; c) WPI-1.5% butter fat (BF); d) CAS-1.5% BF; e) WPI-3% BF; f) CAS-3% BF; g) WPI-1.5% carnauba wax (CW); h) CAS-1.5% CW; i) WPI-3% CW; j) CAS-3% CW.

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This migration into the apple flesh may also have affected the effectiveness of the coating as a barrier to moisture loss and browning.

The continuity of each WPI and CAS-based edible coating when dried on plastic petri plates is illustrated in Figure 7. WPI and CAS-based coatings that contain no lipid seemed to have small particles incorporated into the coating (Fig. 7a, b). These particles could have been protein particles that migrated through the cheesecloth when the coating solutions were filtered. The WPI-based coating with BF appeared to have condensation on the coating surface (Fig. 7c, e) that could have been some butter fat coming out of emulsion. The remaining coatings appeared to be homogeneous (Fig. 7g, i, and Fig. 7d, f, respectively) except CAS-based coatings with CW which showed what might have been solidified wax particles (Fig. 7h, j). The wax may have solidified on contact with the heated coating solutions before homogenization since the coatings were heated to a lower temperature (70°C) than the melting temperature of the CW reported by Stahl and Pitch (82°C). During homogenization, these wax particles may have been homogenized to a size small enough to fit through the cheesecloth filter. These lighter colored areas may also be voids in the protein matrix that the wax filled in.

3.2. Chemical properties of Ida Red and Granny Smith apples

3.2.1 Protein determination of Ida Red and Granny Smith apples

Protein content of Ida Red and Granny Smith apples was evaluated to determine if there was a difference between apple cultivars. Ida Red and Granny Smith apples contained 0.019 and 0.024 mg protein/g apple, respectively, which were similar. Watt and Merrill (1963) reported that apples contain 0.2% protein, which is twofold higher than the

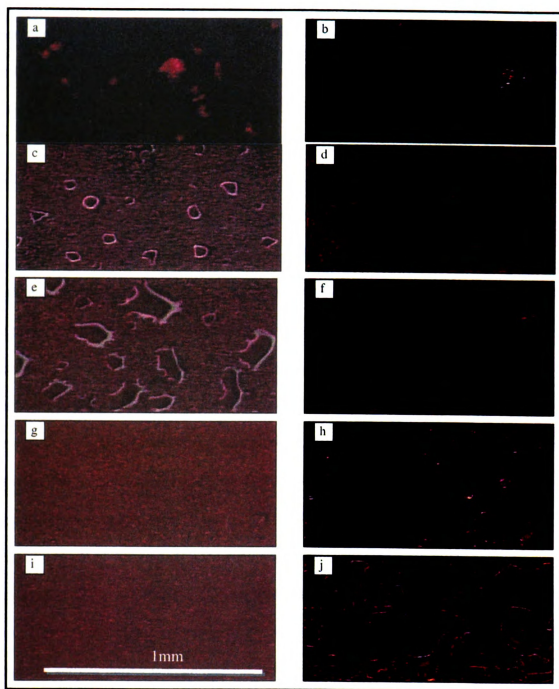


Figure 7. Appearance of coating solutions dried on plastic petri plates shown at 5.5x magnification. a) Whey protein isolate (WPI)-no lipid; b) Lactic acid casein (CAS)-no lipid; c) WPI-1.5% butter fat (BF); d) CAS-1.5% BF; e) WPI-3% BF; f) CAS-3% BF; g) WPI-1.5% carnauba wax (CW); h) CAS-1.5% CW; i) WPI-3% CW; j) CAS-3% CW.

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results of this study. The inconsistency in the present study may be due to human and experimental error. The protein of the apple flesh may not have been completely dissolved into the solution before filtering, therefore the undissolved protein would not be present in the extract.

3.2.2 PPO activity of Ida Red and Granny Smith apples

PPO activity was determined to find a relationship between the PPO activity and the degree of browning in each apple cultivar. PPO activity of Ida Red apples was 2.32 Abs/min/g, which was higher ($p < 0.05$) than for Granny Smith apples (1.39 Abs/min/g). It has been shown that the degree of browning was dependent on presence, concentration and the activity of PPO (Coseteng and Lee, 1987; McEvily et al., 1992; Sapers, 1993). The degree of browning in the Granny Smith and Ida Red apple halves in this study corresponded to the PPO activity in each cultivar, which also is consistent with the results in the literature. PPO activity has been reported to be cultivar dependent (Walker, 1962; Ingle and Hyde, 1968; Vamos-Vigyazo et al., 1976; Coseteng and Lee, 1987), and can differ as a result of different ethylene production and respiration rates of apples after harvest. Brackman and Streif (1994) reported that Granny Smith apples had the lower respiration rate and aroma volatiles, and produced lower ethylene levels due to their low metabolic activity during storage than Ida Red apples.

3.3. Barrier properties of milk protein-based edible coatings

Upon observing the dried coatings microscopically, one would expect the WPI-based coatings containing CW (Fig.7 g, i) and the CAS-based coatings with BF (fig. 7 d, f) to be most effective as barriers because of the apparent homogeneity of the coating.

3.3.1 Total moisture loss of coated and uncoated apple halves

Total moisture loss of coated and uncoated Ida Red and Granny Smith apple halves is shown in Figures 8 a and b and 9 a and b, respectively. Overall, as expected the uncoated controls for both apples and coatings lost the most moisture. All of the WPI and CAS-based edible coatings reduced moisture loss of Ida Red and Granny Smith apple halves. The most effective coating to reduce moisture loss on Ida Red was not the most effective on Granny Smith. CAS-based coatings applied to Ida Red apple halves allowed less moisture loss ($p<0.05$) than the uncoated control (figure 8b). Moisture losses within the apple halves coated with CAS-based coatings were similar. In the case of WPI-based coatings, only coatings containing 1.5 and 3% BF and no lipid allowed less moisture loss ($p<0.05$) compared to the uncoated controls for Ida Red apple halves (figure 8a). The moisture losses of Ida Red apple halves coated with WPI-based coating containing 1.5 and 3% CW were similar to the uncoated controls. All Granny Smith apple halves coated with CAS-based coatings had less moisture loss ($p<0.05$) than the uncoated control except the apple halves coated with CAS-based coating with no lipid (figure 9a). All WPI-based coatings applied to Granny Smith allowed less moisture loss ($p<0.05$) compared to the uncoated control. Moisture losses of the coated Ida Red and Granny Smith apple halves coated with the same protein type were similar.

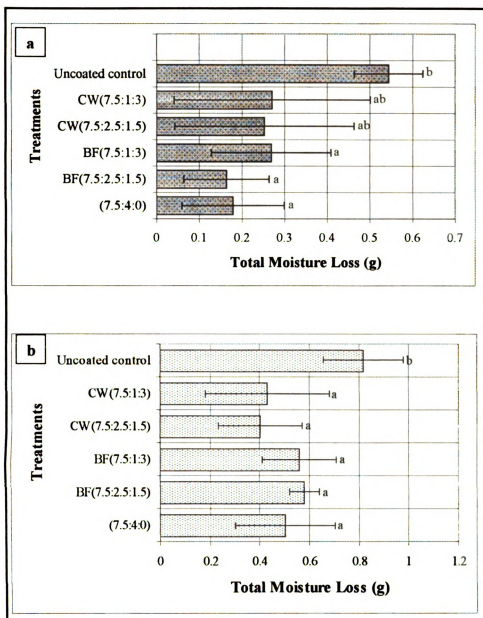


Figure 8. Total moisture loss of Ida Red apple halves coated with milk protein-based edible coatings (@ 4°C, 80% RH). a) WPI-based; b) CAS-based; BF= butter fat, CW= carnauba wax; WPI= whey protein isolate, CAS= lactic acid casein; (Protein:Plasticizer:Lipid).

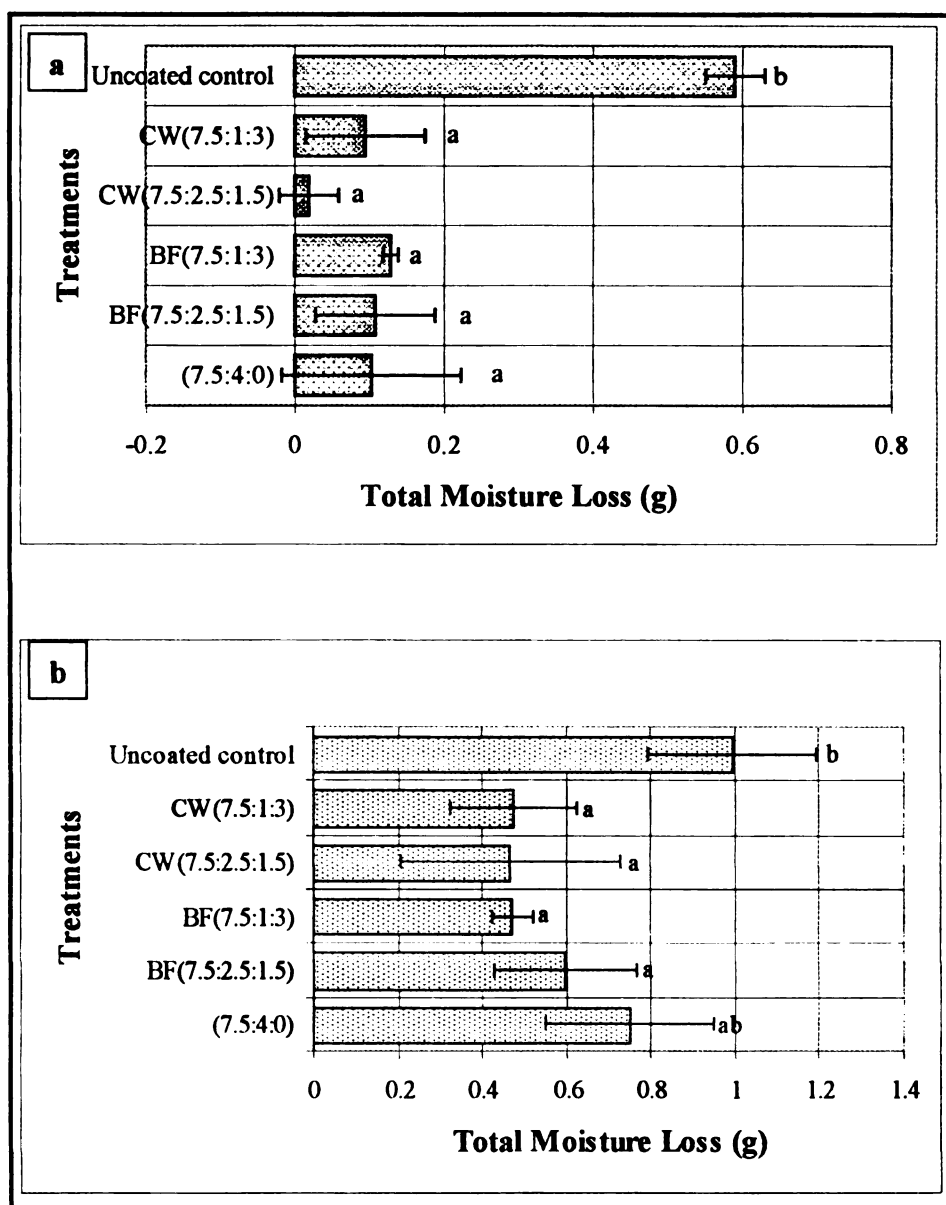


Figure 9. Total moisture loss of Granny Smith apple halves coated with milk protein-based edible coatings (@ 4°C, 80% RH). a) WPI-based; b) CAS-based; BF= butter fat, CW= carnauba wax; WPI= whey protein isolate, CAS= lactic acid casein; (Protein:Plasticizer:Lipid).

Moisture loss of the coated Ida Red and Granny Smith apple halves was not affected by apparent coating thickness. The Granny Smith apple halves coated with WPI-based coatings lost half as much moisture as the Ida Red apple halves coated with the same coatings. Granny Smith apple halves coated with CAS-based coatings lost as much moisture as Ida Red apple halves coated with the same coatings. CAS-coated apple halves may have lost more moisture than the WPI-coated apple halves before storage due to the longer drying time and exposure to the air before storage.

The moisture loss data for both Ida Red and Granny Smith apple halves was highly variable within the same coating treatment. This variability within coated and uncoated apple halves could be attributed to variable respiration rates within the Granny Smith and Ida Red apple cultivars, which may depend on how long the apples were in storage before they were purchased. Variability in the results may also be due to slight variations in the temperature and R.H. from day to day when samples were prepared.

The moisture loss results of this study supported previous results reported by Wong et al. (1994). Water loss of cut apples coated with acetylated monoglyceride and Avicel, alginate, carrageenan or pectin bilayer coatings were recorded over time. They reported the water loss data as a rate and incorporated it in the water vapor resistance (WVR) calculations. All of the coatings in Wong et al. increased WVR compared to the uncoated control, which are inversely proportional to water loss. Therefore, all coated apple samples experienced less water loss than the uncoated controls in that study. Krochta et al. (1990a) reported the moisture loss of sliced apples coated with casein/acetylated monoglyceride emulsions treated with a buffer. They reported that the coatings reduced moisture loss by 50-70% depending on composition and thickness of the

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coating. WPI-based coatings in this study reduced moisture loss of Ida red slices by 40 to 60% and by 85 to 95% for Granny Smith which were similar to the results of Krochta et al. (1990a). The CAS-based coatings in this study reduced moisture loss by 25 to 55% for both Ida Red and Granny Smith apple halves which was less than the results reported by Krochta et al. for casein/acetylated monoglyceride coatings.

Park et al. (1994c) reported that corn zein coatings containing 520 g, 260 g, and 130 g of ethanol as the solvent decreased weight loss of whole tomatoes. They found the coating containing 130 g ethanol most effective, and the coating containing 520 g ethanol least effective. They also reported that the samples coated with the coating containing 130 g ethanol induced fermentation. In another study, Park et al. (1994b) coated whole tomatoes with corn zein coatings containing 260 g ethanol. The coating reduced weight loss and did not induce fermentation. Stuchell and Krochta (1995) reported that WPI and WPI/ Myvacet™ (composed of acetyl monoglycerides) emulsion coatings did not decrease moisture loss of frozen King salmon relative to the uncoated control. However, they reported that the water barrier properties improved if applied as a bilayer coating. Lerdthanangkul and Krochta (1996) reported that WPI, sodium caseinate (SC), and SC/beeswax coatings were ineffective as moisture barriers on whole green peppers. Mineral oil coatings, however significantly reduced moisture loss and the green peppers remained marketable. These studies show that coating effectiveness depends on the food being coated. Our study took these results a step further and determined that coatings were effective moisture and gas barriers on minimally processed produce such as sliced fruit. However, we also found that the effectiveness of coatings were cultivar dependent.

3.3.2 Water vapor resistance of the coated and uncoated apple halves

Water vapor resistance (WVR) has been defined as a measure of how efficiently the coating resists moisture migration. A good barrier to moisture would have a high WVR value. Figures 10a and b and 11a and b show the WVRs of the WPI and CAS-based coatings applied to Ida Red and Granny Smith apple halves, respectively. The WVR of the WPI-based coating with 3% CW applied to Granny Smith halves was calculated from only 3 values because the fourth was an outlier (Figure 11a).

WPI-based coatings applied to Ida Red apple halves had higher WVRs ($p < 0.05$) than the uncoated controls except apple halves coated with 3% lipid (figure 10b). These coated apple halves also had a higher degree of variability, which could be due to an excessive disruption of the protein matrix by the high percentage of lipid added to the coating solution (Fig 7j) or the apparent separation of the lipid from the coating (Fig. 7e). CAS-based coatings, however, were not as efficient as WPI-based coatings in decreasing moisture migration on Ida Red apple halves except the coating containing 1.5% CW, which had a higher ($p < 0.05$) WVR than the uncoated control (Fig. 10a and b). The WPI-based coatings applied to the Granny Smith apple halves had the most favorable WVRs, which were higher ($p < 0.05$) than the uncoated control except for the coating with 3% CW (Figure 11a). The CAS-based coatings were not as efficient, however, the WVRs were higher ($p < 0.05$) than the uncoated control except for the coatings containing butter fat (Figure 11b).

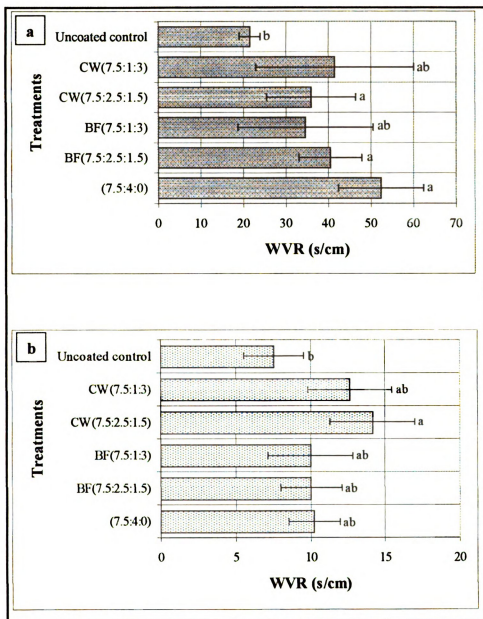


Figure 10. Water vapor resistance (WVR) of Ida Red apple halves coated with milk protein-based edible coatings (@4°C, 80% RH). a) WPI-based; b) CAS-based; BF= butter fat, CW= carnauba wax; WPI= whey protein isolate; CAS= lactic acid casein; (Protein:Plasticizer:Lipid)

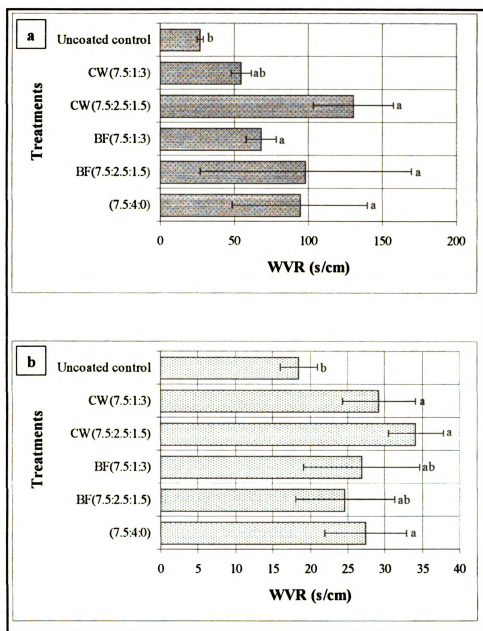


Figure 11. Water vapor resistance (WVR) of Granny Smith apple halves coated with milk protein-based edible coatings (@ 4°C, 80% R.H.). a) WPI-based; b) CAS-based; BF= butter fat, CW= carnauba wax, WPI= whey protein isolate; CAS= lactic acid casein; (Protein:Plasticizer:Lipid)

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These results should have shown a similar trend as the moisture-loss rate (MLR) data shown in Appendix 2, which was used to calculate the WVR. There was much variability in these results due to a magnified effect from incorporating the MLR into the equation. Some of the WVR results lost their significance due to the variability in the MLRs. The WVRs of the WPI and CAS-based coatings applied to Granny Smith were higher than when the same coatings were applied to Ida Red apple halves.

Avena-Bustillos et al. (1993) reported that sodium caseinate/stearic acid coatings in ratios of 1:3, 1:1, and 1:2 significantly increased the WVR of the coatings on carrots. The results of Avena-Bustillos' study were similar to the CAS-based coatings applied to Ida Red apple halves, but were worse than the CAS-based coatings applied to Granny Smith apple halves, and much worse than the WPI-based coating results of both apple cultivars in the present study. In a later study, Avena-Bustillos et al. (1994a) reported that sodium caseinate films with beeswax and stearic acid, as well as calcium caseinate coatings with beeswax, stearic acid, and acetylated monoglyceride, increased ($p < 0.05$) the WVR of the coating. Both studies reported WVRs that were similar to the results of the CAS-based coatings applied to Ida Red apple halves in our study. The WVRs for calcium caseinate coatings and Semperfresh coatings applied to zucchini (18 to 24 sec/cm) (Avena-Bustillos et al., 1994b) were better than the CAS-based coatings applied to Ida Red apple halves (10.00 to 14.15 sec/cm) in this study. However, they were worse than the WVRs of the CAS-based coatings applied to Granny Smith apple halves (24.66 to 34.18 sec/cm). The WVR results for WPI-based coatings applied to both Ida Red (34.65 to 52.4 sec/cm) and Granny Smith apple halves (54.81 to 130.2 sec/cm) were better than

the WVRs in Avena-Bustillos et al. (1994b). The WVRs of calcium caseinate coatings in an optimization study were similar (30 to 42 sec/cm) to WPI-coating results in this study. Wong et al. (1994) coated cut apple pieces by dipping them first in an acid/calcium solution, and then in one of four different polysaccharide/acetylated monoglyceride coating solutions. They reported that all four coatings significantly increased the WVR of the apple pieces. The results of Wong et al. (1994) were similar to the WVR results for WPI-based coatings in the present study for the Ida Red apple halves.

3.3.3 Browning of coated and uncoated Ida Red and Granny Smith apple halves and internal oxygen content

3.3.3.1 Colorimeter determination of browning. Difference in L-value of coated and uncoated Ida Red and Granny Smith apple halves are illustrated in figures 12a and b and 13a and b, respectively. As expected, coated and uncoated Ida Red apple halves had larger differences in L-values or more browning than coated and uncoated Granny Smith apple halves. These results were consistent with the PPO results presented in section 3.2.2. None of the CAS or WPI-based coatings applied to Ida Red apple halves decreased the degree of browning of the apple halves compared to the uncoated controls except the WPI-based coating containing 3% CW which decreased ($p<0.05$) browning compared to the uncoated control (Figure 12a and b). The degree of browning within the CAS and WPI-based coated Ida Red apple halves was similar. All of the WPI-based edible coatings applied to Granny Smith apple halves reduced browning of the apple halves ($p<0.05$), whereas the CAS-based coated apple halves had a similar degree of browning

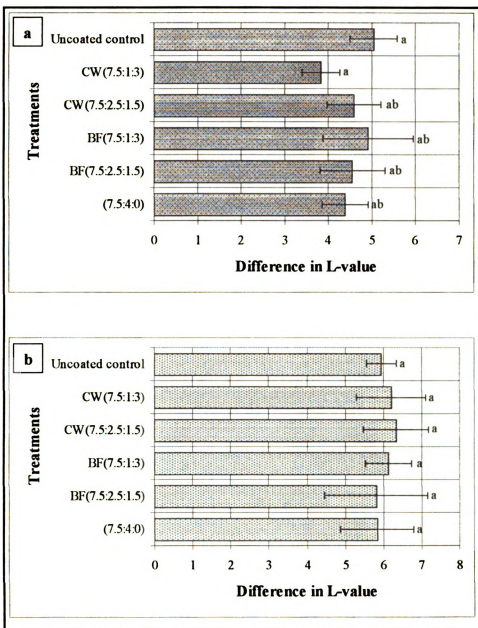


Figure 12. Difference in L-value of Ida Red apple halves coated with milk protein-based edible coatings (@ 4°C, 80% R.H.). a) WPI-based; b) CAS-based; BF= butter fat, CW= carnauba wax; WPI= whey protein isolate; CAS= lactic acid casein; (Protein:Plasticizer:Lipid)

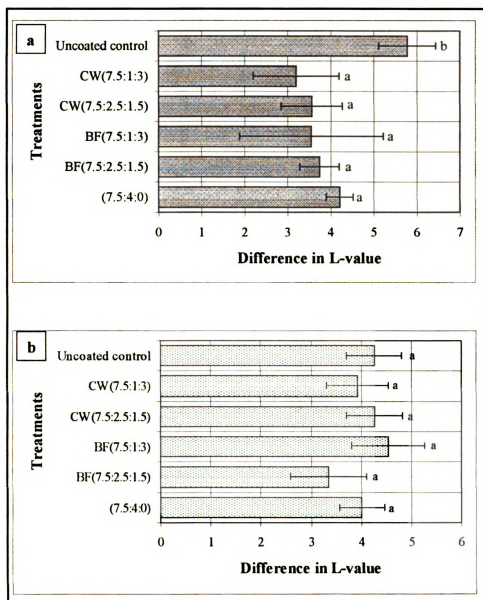


Figure 13. Difference in L-value of Granny Smith apple halves coated with milk protein-based edible coatings (@ 4°C, 80% R.H.). a) WPI-based; b) CAS-based; BF= butter fat, CW= carnauba wax, WPI= whey protein isolate; CAS= lactic acid casein; (Protein:Plasticizer:Lipid)

compared to the uncoated control (Figure 13a and b). The degree of browning within the CAS and WPI-based coated Granny Smith apple halves was similar.

The colorimeter results showed that coated and uncoated Ida Red apple halves browned to a higher degree than the Granny Smith apple halves. It was expected that the coatings would limit the oxygen flow into the coated apple halves. However, this was not the case, as determined by the results of the internal oxygen content experiment as it is reported in section 3.3.4.2. Rolle and Chism (1987) reported that calcium played a role in maintaining cell wall structure in fruits by interacting with pectic acid in the cell walls, which forms calcium pectate. It also has been reported that the presence of calcium was important in maintaining cell permeability and compartmentation, and that fruits deficient in calcium experience PPO induced enzymatic browning (Wienke, 1980). The calcium contained in the milk protein-based coatings developed in this study may have been enough to repair the cell walls and disrupt the browning reaction induced by the release of PPO when the apple halves were cut, which decrease the degree of browning of the apple halves.

Most research up to now has focused on the effects of coatings on interior and exterior color change and ripening of coated whole apples. With the convenience of sliced fruit in mind, this study looked at the effect coatings applied to the cut apple flesh has on the browning reaction caused by the minimal processing.

Santerre et al. (1989) determined the internal color change of whole McIntosh, Golden Delicious, apples coated with various levels of Semperfresh coating. Internal color change of both apple cultivars was not affected ($p < 0.05$) by Semperfresh at any level. Any color change that did occur was attributed to storage and maturity of the

apples (Santerre et al., 1989). Chai et al. (1991) coated whole Ida Red, McIntosh, and Golden Delicious apples with Semperfresh based coatings at various concentrations. They reported cultivar differences when evaluating the effect of Semperfresh on color change of the internal flesh of the whole fruits. The flesh of Ida Red apples were not affected by Semperfresh coatings, whereas the McIntosh and Golden Delicious were significantly affected Semperfresh concentration and/or storage interval (Chai et al., 1991). The flesh of McIntosh and Golden Delicious samples increased in brightness during the first two months, however, McIntosh flesh brightened faster than Golden Delicious. Both apple cultivars began to lose brightness during the next three months at the same rate.

3.3.3.2 Interior oxygen content of coated and uncoated apple halves. Air normally contains 21.7% oxygen at 1.0 atm. Once an apple is picked, it respire through the skin. Disruption of this process by cutting affects the rate of respiration. Edible coatings could decrease the rate of respiration by forming a barrier to oxygen on the surface of a cut apple. If the respiration rate decreases enough, ethylene production and enzymatic browning would be decreased. This study measured the interior oxygen content of coated and uncoated cut apples to determine whether the coatings formed a barrier to oxygen on the apple surface.

The three WPI-based coatings that were used for this study were those with 1.5% BF, 1.5% CW, and 3% CW. The two CAS-based coatings that were used for this study were the coating without lipid, and with 1.5% BF. Table 7 a and b shows the steady state interior oxygen levels for WPI and CAS-based coated and uncoated Ida Red and Granny

Table 7. Interior oxygen content of apple halves coated with milk protein-based edible coatings (@ 4°C, 93% R.H).

a)

Interior Oxygen Content (kPa) ^{a,b}		
WPI		
Treatments ^c	Ida Red	Granny Smith
BF (7.5:2.5:1.5)	15.79 ± 0.19 ^d	16.87 ± 0.48 ^d
CW (7.5:2.5:1.5)	16.79 ± 0.75 ^d	17.81 ± 1.07 ^d
CW (7.5:1:3)	16.25 ± 0.94 ^d	17.63 ± 0.64 ^d
Uncoated Control	19.88 ± 0.38 ^e	19.79 ± 0.31 ^e

b)

Interior Oxygen Content (kPa) ^{a,b}		
CAS		
Treatments ^c	Ida Red	Granny Smith
(7.5:4:0)	15.82 ± 0.33 ^d	15.37 ± 2.02 ^d
BF (7.5:2.5:1.5)	15.85 ± 0.94 ^d	15.41 ± 2.18 ^d
Uncoated Control	19.75 ± 0.11 ^e	20.07 ± 0.35 ^e

^a WPI= whey protein isolate, CAS= lactic acid casein.

^b Means ± standard deviation; n=3 for all treatments.

^c BF= butter fat, CW= carnauba wax; (Protein:Plasticizer:Lipid)

^{d-e} columnwise denote significant differences compared to the control (p<0.05).

Smith apple halves, respectively. All coated Ida Red and Granny Smith apple halves had lower ($p < 0.05$) interior oxygen levels than the uncoated controls. The interior oxygen content within the coated Ida Red and Granny Smith apple halves coated with the same protein type was similar.

The steady state levels of interior oxygen in the coated apple halves were not low enough to conclude that it was a lack of oxygen on the flesh surface that slowed the browning reaction. Researchers have reported that the presence of calcium on the cut surface of fruits may decrease ethylene production (Faust, 1975; Leiberman and Wang, 1982) through a degree of oxidative metabolism (Frost, 1986). The presence of calcium also has been reported to reduce respiration rates by limiting substrate diffusion to the respiratory enzymes in the cytoplasm (Bangerth et al., 1972). Since WPI and CAS contain small amounts of calcium, and the interior oxygen levels of the coated samples were not low enough to suppress the browning reaction, the chelating function of these calcium molecules may be retarding the browning reaction, not the effect of the coatings oxygen barrier properties.

McHugh et al. (1993) and Mate et al. (1996) reported that coating thickness had an effect on barrier properties of coatings. The coatings in this study might not have been thick enough to form a good oxygen barrier. Samples in this study were coated a third time to determine if thickness effected the oxygen content. The levels of oxygen decreased to between 12 and 14 kPa. Gran and Beaudry (1993) reported that an internal oxygen level of 2 kPa would be needed to retard browning on the surface. Cut apple pieces coated with polysaccharide/lipid coatings showed a significant decrease (50% to 75%) in internal oxygen, which suggested that they were effective barriers to the diffusion

of oxygen into the apple (Wong et al., 1994). Acetylated monoglyceride has been reported to have very high WVRs in general and on cut apple pieces and therefore slowed respiration (Kester and Fennema, 1989b; Wong et al., 1994). Wong et al. concluded that the low levels of oxygen contributed to a decrease in ethylene production and therefore, slowed respiration. However, they did not report on subsequent degree of browning. In this study, interior oxygen level decreased by only 20%, however browning was still reduced ($p < 0.05$). Avena-Bustillos et al. (1994a) reported that caseinate-based coatings decreased internal oxygen levels of coated and uncoated carrots to 1%. Lerdthanangkul and Krochta (1996) reported that internal oxygen content of green bell peppers coated with WPI, sodium caseinate, sodium caseinate/beeswax, mineral oil, and cellulose based coatings did not differ. Levels in their study ranged from 14 to 19% O₂ (equal to 14 to 19 kPa) for intact fruit, which were similar to the results of this study. The internal oxygen content of corn zein coated whole tomatoes did not differ from the uncoated controls. Smith and Stow (1984) reported an internal oxygen level of 10 to 16% for whole Cox's Orange Pippin apples coated with carboxymethylcellulose-sucrose fatty acid ester coating which were similar to the uncoated controls. This data was similar to the internal oxygen levels for coated and uncoated apple halves in this study.

3.4. Shelf life of coated and uncoated apple halves during 14 days of refrigerated storage

Coating treatments for the storage study were selected based on their superior barrier properties as determined in section 3.3. The two WPI-based coatings chosen were

those containing 1.5% BF or CW. The single best CAS-based coating for decreasing color change used in the storage study was the coating containing 1.5% BF.

3.4.1 Moisture loss determination

The data for moisture loss of Ida Red and Granny Smith apple halves occurring over time is shown in Table 8a and b, respectively. The uncoated controls were cut at the same time the coated samples were dipped and were exposed to the air for the same drying time as the respective coated samples. There was no moisture lost before the coating application as the slices were cut just before they were dipped into the coating solutions. By the end of the drying time the CAS coated Ida Red halves and the uncoated controls of both cultivars lost moisture ($p<0.05$). The WPI coated apple halves of both cultivars gained moisture, which may be due to the WPI-based coatings hydrophilic nature and tendency to rehydrate. All WPI and CAS-based coatings were effective in reducing total moisture loss ($p<0.05$) of both apple cultivars up to 14 days of storage compared to uncoated controls except the CAS-based coating applied to Granny Smith apple halves. The total moisture loss of the coated apple halves for both cultivars were similar. The uncoated Ida Red and Granny Smith apple halves lost more than twice as much moisture as any coated apple halves by the end of storage (Table 8a and b). Ida Red apple halves coated with WPI lost moisture ($p<0.05$) by day 6, whereas the apple halves coated with CAS-BF and the uncoated control lost moisture ($p<0.05$) right after the coating period compared to when they were freshly cut. Granny Smith apple halves coated with WPI lost moisture ($p<0.05$) by day 6, whereas the apple halves coated with CAS-BF lost moisture

Table 8. Total moisture loss of apple halves coated with milk protein-based edible coatings stored over time (@ 4°C, 80% RH).

a)

Total Moisture Loss (g) ^{a,b}				
Ida Red				
Treatments ^c				
Storage (Days)	WPI-BF (7.5:2.5:1.5)	WPI-CW (7.5:2.5:1.5)	CAS-BF (7.5:2.5:1.5)	Uncoated control
Fresh Cut	0.00 ^{d,1}	0.00 ^{d,1}	0.00 ^{d,1}	0.00 ^{d,2}
After Coating	0.23 ± 0.15 ^{e,1*}	0.17 ± 0.29 ^{dc,1*}	0.20 ± 0.12 ^{e,1}	1.00 ± 0.17 ^{e,2}
2	0.13 ± 0.21 ^{dc,1}	0.15 ± 0.29 ^{dc,1}	0.49 ± 0.26 ^{e,1}	1.25 ± 0.22 ^{e,2}
4	0.21 ± 0.22 ^{dc,1}	0.26 ± 0.29 ^{dc,1}	0.54 ± 0.33 ^{e,1}	1.32 ± 0.25 ^{e,2}
6	0.26 ± 0.23 ^{e,1}	0.32 ± 0.29 ^{e,1}	0.61 ± 0.35 ^{e,1}	1.37 ± 0.27 ^{e,2}
8	0.33 ± 0.24 ^{e,1}	0.38 ± 0.31 ^{e,1}	0.66 ± 0.33 ^{e,1}	1.40 ± 0.27 ^{e,2}
10	0.36 ± 0.25 ^{e,1}	0.41 ± 0.30 ^{e,1}	0.68 ± 0.34 ^{e,1}	1.42 ± 0.28 ^{e,2}
12	0.40 ± 0.26 ^{e,1}	0.46 ± 0.30 ^{e,1}	0.72 ± 0.34 ^{e,1}	1.45 ± 0.30 ^{e,2}
14	0.42 ± 0.25 ^{e,1}	0.48 ± 0.29 ^{e,1}	0.75 ± 0.34 ^{e,1}	1.48 ± 0.31 ^{e,2}

b)

Total Moisture Loss (g) ^{a,b}				
Granny Smith				
Treatments ^c				
Storage (Days)	WPI-BF (7.5:2.5:1.5)	WPI-CW (7.5:2.5:1.5)	CAS-BF (7.5:2.5:1.5)	Uncoated control
Fresh Cut	0.00 ^{d,1}	0.00 ^{d,1}	0.00 ^{d,1}	0.00 ^{d,1}
After Coating	0.11 ± 0.34 ^{dc,1*}	0.23 ± 0.20 ^{e,1*}	0.28 ± 0.28 ^{dc,1}	1.21 ± 0.40 ^{e,2}
2	0.29 ± 0.38 ^{dc,1}	0.17 ± 0.27 ^{dc,1}	0.61 ± 0.37 ^{e,1}	1.42 ± 0.36 ^{e,2}
4	0.40 ± 0.40 ^{dc,1}	0.26 ± 0.30 ^{dc,1}	0.70 ± 0.39 ^{e,1}	1.48 ± 0.34 ^{e,2}
6	0.46 ± 0.40 ^{e,1}	0.33 ± 0.32 ^{e,1}	0.76 ± 0.39 ^{e,1}	1.55 ± 0.30 ^{e,2}
8	0.52 ± 0.41 ^{e,1}	0.40 ± 0.35 ^{e,1}	0.81 ± 0.40 ^{e,1}	1.57 ± 0.32 ^{e,2}
10	0.55 ± 0.43 ^{e,1}	0.44 ± 0.38 ^{e,1}	0.86 ± 0.41 ^{e,12}	1.58 ± 0.32 ^{e,2}
12	0.60 ± 0.42 ^{e,1}	0.48 ± 0.37 ^{e,1}	0.90 ± 0.42 ^{e,12}	1.61 ± 0.32 ^{e,2}
14	0.62 ± 0.44 ^{e,1}	0.51 ± 0.40 ^{e,1}	0.94 ± 0.43 ^{e,12}	1.64 ± 0.32 ^{e,2}

^a WPI = whey protein isolate-based; CAS = lactic acid casein-based.

^b Means ± standard deviation; n=3 for all treatments.

^c BF=butter fat, CW=carnauba wax; (Protein:Plasticizer:Lipid)

^{*} Samples gained weight after coating.

^{d-e} Columnwise denotes significant difference over time (p<0.05).

¹⁻² Row-wise denote significant differences (p<0.05).

($p < 0.05$) by day 2, and the uncoated control lost moisture ($p < 0.05$) right after the coating period compared to when they were fresh cut. All coated and uncoated apple halves of both cultivars continued to lose moisture after drying throughout the study compared to when they were fresh cut. However, statistically the moisture lost between each reading throughout the study was not significant due to the large variability among apple halves at each data collection as discussed previously in section 3.3.1. The results for moisture loss of coated and uncoated apple halves in this storage study exhibit the same trends as the moisture loss data discussed in section 3.3.1 compared to the uncoated control.

3.4.2. WVR of coated and uncoated apple halves after 14 days of refrigerated storage

The WVRs of WPI and CAS-based edible coatings applied to Ida Red and Granny Smith apple halves are shown in Table 9. The WVR was calculated with the WLR shown in Table 14 in appendix 2. The MLRs were calculated as an average over the 14 day storage period, therefore the WVR data was calculated as an average over time.

However, as MLRs decrease over time, the WVR increased proportionally to the decrease in rate. The average WVRs after 14 days of refrigerated storage for all of the coatings applied to Ida Red and Granny Smith apple halves were higher compared to the uncoated controls. However, these differences were not statistically significant due to large variability in the data. The WVRs for the WPI-based coated apple halves in the storage study were lower than the previous results reported in section 3.3.2, which may be due to the longer duration of the storage study. The moisture loss between readings decreased over the duration of the storage study. MLRs of the samples were calculated as an average over time, therefore the WVR may not reflect this rate change, thus the WVR

results may be lower than was expected. The WVR of the CAS-based coating applied to Ida Red apple halves was higher than the WVR of the same coating applied to Granny Smith apple halves which was contrary to the previous results. The WVRs of WPI coatings of the same cultivar were similar to those of the CAS coating, whereas in previous results they were much higher. The degree of variability in the storage study was similar to that of the previous WVR results and the source of variability may be the same as previously discussed.

Table 9. Water vapor resistance (WVR) of milk protein-based edible coatings on apple halves after 14 days of refrigerated storage (@ 4°C, 80% RH).

Water Vapor Resistance (s/cm)^{a,b}		
Treatments^c	Ida Red	Granny Smith
WPI-BF (7.5:2.5:1.5)	20.84 ± 10.20	19.64 ± 10.80
WPI-CW (7.5:2.5:1.5)	18.69 ± 8.66	21.69 ± 12.90
CAS-BF (7.5:2.5:1.5)	20.26 ± 13.80	16.54 ± 8.15
Uncoated control	11.38 ± 6.11	12.50 ± 6.12

^a WPI = whey protein isolate; CAS= lactic acid casein.

^b Means ± standard deviation; n=3 for all treatments. No significant differences were observed.

^c BF= butter fat, CW= carnauba wax; (Protein:Plasticizer:Lipid)

3.4.3. Browning of apple halves during refrigerated storage as determined by a colorimeter

Browning of Ida Red and Granny Smith apple halves during 14 days of refrigerated storage is reported in Table 10 a and b, respectively. Coated and uncoated Granny Smith apple halves browned to a lesser degree than Ida Red apple halves throughout the storage period. All coated and uncoated apple halves turned brown ($p < 0.05$) compared to when they were fresh cut, which was consistent with the PPO activity in these apple cultivars as reported previously in section 3.2.2. The coated and uncoated apple halves for both cultivars continued to increase in degree of browning throughout the study. Before day 6, Ida Red apple halves coated with WPI were less brown ($p < 0.05$) than the uncoated control. The degree of browning of the Ida Red apple halves coated with CAS-BF was similar to the uncoated control throughout the study. The degree of browning for coated Granny Smith apple halves was less ($p < 0.05$) than the uncoated control until day 4 and day 6 for WPI-BF and WPI-CW coated apple halves, respectively. The degree of browning for Granny Smith apple halves coated with CAS-BF was similar to the uncoated control throughout the study except on day 2 when it was less ($p < 0.05$). After coating, the degree of browning of Ida Red halves coated with WPI-BF and WPI-CW increased ($p < 0.05$) by day 2 and day 6, respectively, whereas the apple halves coated with CAS and the uncoated controls increased ($p < 0.05$) in degree of browning by day 10 and day 4, respectively. The degree of browning of all coated Granny Smith apple halves increased ($p < 0.05$) by day 6, whereas the browning of the uncoated controls increased

Table 10. Difference in L-values of apple halves coated with milk protein-based edible coatings during 14 days of refrigerated storage (@ 4°C, 80% R.H.).

a)

Difference in L-value ^{a,b}				
Ida Red				
Treatments ^c				
Storage (Days)	WPI-BF (7.5:2.5:1.5)	WPI-CW (7.5:2.5:1.5)	CAS-BF (7.5:2.5:1.5)	Uncoated control
Fresh Cut	0.00 ^{d,1}	0.00 ^{d,1}	0.00 ^{d,1}	0.00 ^{d,1}
After Coating	3.08 ± 0.09 ^{e,1}	2.69 ± 0.47 ^{e,1}	4.53 ± 0.66 ^{e,2}	4.55 ± 0.37 ^{e,2}
2	3.66 ± 0.36 ^{f,1,2}	3.43 ± 0.72 ^{e,2}	4.71 ± 0.74 ^{e,1,2}	4.97 ± 0.29 ^{e,1}
4	3.90 ± 0.24 ^{g,1}	3.95 ± 0.58 ^{e,1}	4.75 ± 0.86 ^{e,1,2}	5.18 ± 0.32 ^{f,2}
6	4.53 ± 0.47 ^{gh,1}	4.96 ± 0.36 ^{g,1}	5.45 ± 0.83 ^{e,1}	5.73 ± 0.59 ^{g,1}
8	5.33 ± 0.43 ^{i,1}	5.60 ± 0.60 ^{gh,1}	6.01 ± 0.82 ^{e,1}	6.15 ± 0.50 ^{g,1}
10	6.01 ± 0.43 ^{i,1}	6.36 ± 0.60 ^{h,1}	6.63 ± 0.87 ^{g,1}	6.61 ± 0.39 ^{gh,1}
12	6.47 ± 0.20 ^{jk,1}	7.06 ± 0.72 ^{j,1}	7.16 ± 0.77 ^{g,1}	7.37 ± 0.50 ^{h,1}
14	7.06 ± 0.25 ^{l,1}	7.70 ± 0.84 ^{k,1}	7.88 ± 0.85 ^{g,1}	7.74 ± 0.48 ^{i,1}

b)

Difference in L-value ^{a,b}				
Granny Smith				
Treatments ^c				
Storage (Days)	WPI-BF (7.5:2.5:1.5)	WPI-CW (7.5:2.5:1.5)	CAS-BF (7.5:2.5:1.5)	Uncoated control
Fresh Cut	0.00 ^{d,1}	0.00 ^{d,1}	0.00 ^{d,1}	0.00 ^{d,1}
After Coating	1.14 ± 0.32 ^{g,1}	1.00 ± 0.65 ^{e,1}	1.98 ± 0.11 ^{e,1,2}	2.51 ± 0.68 ^{e,2}
2	1.94 ± 0.45 ^{g,1}	1.74 ± 0.71 ^{e,1}	2.38 ± 0.22 ^{e,1}	3.62 ± 0.55 ^{g,2}
4	2.27 ± 0.72 ^{eh,1,2}	2.30 ± 0.90 ^{e,1,2}	2.74 ± 0.29 ^{e,1,2}	3.88 ± 0.32 ^{g,1}
6	3.46 ± 1.10 ^{gh,1}	3.87 ± 1.10 ^{g,1}	4.01 ± 0.34 ^{f,1}	4.79 ± 0.60 ^{gh,1}
8	4.11 ± 1.20 ^{h,1}	4.96 ± 1.30 ^{gh,1}	4.88 ± 0.28 ^{g,1}	5.40 ± 0.71 ^{h,1}
10	4.68 ± 1.20 ^{hi,1}	5.79 ± 1.20 ^{gh,1}	5.66 ± 0.59 ^{h,1}	5.80 ± 0.73 ^{h,1}
12	5.14 ± 1.30 ^{i,1}	6.23 ± 1.30 ^{gh,1}	6.03 ± 0.67 ^{h,1}	6.22 ± 0.61 ^{h,1}
14	5.57 ± 1.40 ^{i,1}	6.64 ± 1.20 ^{h,1}	6.43 ± 0.62 ^{h,1}	6.33 ± 0.78 ^{h,1}

^a WPI= whey protein isolate-based; CAS= lactic acid casein-based.

^b Means ± standard deviation; n=3 for all treatments.

^c BF= butter fat, CW= carnauba wax; (Protein:Plasticizer:Lipid)

^{d-1} columnwise denote significant differences (p<0.05) over time.

¹⁻² Row-wise denote significant differences (p<0.05).

($p < 0.05$) by day 2. After 3 days of storage, the colorimeter results for CAS coated and uncoated Ida Red and Granny Smith apple halves were consistent, whereas the WPI coated apple halves were inconsistent with the results discussed previously in section 3.3.3.1.

3.4.4. Visual panel evaluation of browning of coated and uncoated apple halves

Visual panel results for the degree of browning of coated and uncoated Ida Red and Granny Smith apple halves are shown in Figure 11 a and b, respectively. The visual panel was discontinued after day 6 due to the deterioration of the coated and uncoated apple halves, thus, they were no longer considered acceptable by the panelists. Coated and uncoated Granny Smith halves were generally scored less brown than the Ida Red halves throughout the study. However, the trends discussed apply to both cultivars unless otherwise stated. Panel results were consistent with the colorimeter results in section 3.4.3. On day 0, panelists were given coated apple halves as soon as the coatings were dried, and the uncoated halves were given to them freshly cut. This was done to assimilate what they would see as “fresh” slices on a salad bar or catered event. On day 0 the apple halves coated with WPI were scored less brown ($p < 0.05$) than the CAS coated apple halves. The uncoated controls were scored as nearly fresh cut, which was expected, however, by day 2, the uncoated controls were scored more brown ($p < 0.05$) than on day 0. After day 2, the uncoated controls scored very brown. The CAS coated apple halves were scored brown throughout the study. The brownness of apple halves coated with WPI-BF and WPI-CW were similar throughout the study except for Ida Red apple halves on day 4 when WPI-BF coated apple halves scored less brown ($p < 0.05$). The Ida Red

Table 11. Effect of storage time on browning of apple halves coated with milk protein-based edible coatings as determined by a trained sensory panel (@ 4°C, 80% RH).

a)

Browning^{a,b}				
Ida Red				
Treatments^c	Day 0	Day 2	Day 4	Day 6
WPI-BF(7.5:2.5:1.5)	4.94 ^{d,1}	5.79 ^{d,1}	7.00 ^{d,2}	7.85 ^{d,2}
WPI-CW(7.5:2.5:1.5)	5.42 ^{d,1}	6.40 ^{de,1}	7.55 ^{e,1}	8.21 ^{d,1}
CAS-BF(7.5:2.5:1.5)	8.24 ^{e,1}	7.55 ^{ef,1}	7.76 ^{e,1}	8.30 ^{d,1}
Uncoated control	1.21 ^{f,1}	8.06 ^{f,2}	8.42 ^{e,2}	8.70 ^{e,2}

b)

Browning^{a,b}				
Granny Smith				
Treatments^c	Day 0	Day 2	Day 4	Day 6
WPI-BF(7.5:2.5:1.5)	2.91 ^{d,1}	3.76 ^{d,1}	6.85 ^{d,2}	7.18 ^{d,3}
WPI-CW(7.5:2.5:1.5)	3.03 ^{d,1}	4.67 ^{d,2}	7.18 ^{d,3}	7.85 ^{d,4}
CAS-BF(7.5:2.5:1.5)	5.94 ^{e,1}	6.24 ^{e,1}	7.82 ^{d,1}	8.18 ^{d,1}
Uncoated control	1.30 ^{f,1}	7.97 ^{e,2}	7.70 ^{d,2}	8.09 ^{d,2}

^a WPI = whey protein isolate; CAS = lactic acid casein.

^b Means reported are the least square means for day and treatment $\pm$.01 (the SEM for Ida Red samples) or .02 (the SEM for Granny Smith samples); Scores given to each treatment over time using an hedonic scale of 1-9 with 1=fresh cut, and 9=very brown; n=33 (3 replicates x 11 judges).

^c BF=butter fat, CW=carnauba wax. (Protein:Plasticizer:Lipid)

^{d-f} Columnwise denote significant differences ($p<0.05$).

¹⁻⁴ Row-wise denote significant differences over time ($p<0.05$).

apple halves coated with WPI-BF were scored less brown ($p < 0.05$) than the uncoated controls throughout the study. Granny Smith apple halves coated with WPI-BF scored less brown ($p < 0.05$) than the uncoated control until day 4. Granny Smith apple halves coated with WPI-BF and WPI-CW were scored increasingly more brown ($p < 0.05$) after day 2 and day 0, respectively throughout the study. The variability among individual panelists, in addition to the variability among samples of the same cultivar and coating type may have affected the significance of these results.

3.4.5. Visual panel overall acceptance of coated and uncoated apple halves

The visual panel members also scored the same coated and uncoated Ida Red and Granny Smith apple halves with regard to overall acceptance over time. These results are shown in Table 12 a and b, respectively. Trends discussed in this section apply to both cultivars unless otherwise stated. Trends for overall acceptance of uncoated controls and CAS coated apple halves throughout the study were similar to those discussed in section 3.4.4. On day 0, the uncoated controls were very acceptable and better ($p < 0.05$) than all coated samples, however by day 2 the uncoated controls were unacceptable ($p < 0.05$). Coated and uncoated Granny Smith apple halves were generally more acceptable than Ida Red apple halves. WPI coated apple halves were more acceptable ($p < 0.05$) than the uncoated controls until day 4. WPI-BF coated apple halves were more acceptable on any day of data collection. By day 4, all coated and uncoated apple halves were unacceptable. The WPI-BF and WPI-CW coated apple halves were similarly acceptable throughout the study. Granny Smith apple halves coated with WPI-based coatings were acceptable until day 2 and were increasingly unacceptable ($p < 0.05$) thereafter. The WPI-BF and WPI-CW

Table 12. Overall acceptance of apple halves coated with milk protein-based edible coatings as determined by a sensory panel.

a)

Overall Acceptance ^{a,b}				
Ida Red				
Treatments ^c	Day 0	Day 2	Day 4	Day 6
WPI-BF(7.5:2.5:1.5)	2.76 ^{d,1}	2.39 ^{d,1}	1.82 ^{d,2}	1.52 ^{d,3}
WPI-CW(7.5:2.5:1.5)	2.76 ^{d,1}	2.09 ^{d,2}	1.67 ^{d,3}	1.33 ^{d,4}
CAS-BF(7.5:2.5:1.5)	1.42 ^{e,1}	1.79 ^{de,1}	1.64 ^{d,1}	1.30 ^{d,1}
Uncoated control	4.88 ^{f,1}	1.46 ^{e,2}	1.39 ^{d,2}	1.18 ^{d,2}

b)

Overall Acceptance ^{a,b}				
Granny Smith				
Treatments ^c	Day 0	Day 2	Day 4	Day 6
WPI-BF(7.5:2.5:1.5)	3.94 ^{d,1}	3.33 ^{d,1}	1.94 ^{d,2}	1.88 ^{d,3}
WPI-CW(7.5:2.5:1.5)	3.88 ^{d,1}	3.03 ^{d,1}	1.88 ^{d,2}	1.46 ^{d,3}
CAS-BF(7.5:2.5:1.5)	2.48 ^{e,1}	2.30 ^{de,1}	1.67 ^{d,1}	1.30 ^{d,1}
Uncoated control	4.88 ^{f,1}	1.64 ^{e,2}	1.49 ^{d,2}	1.45 ^{d,2}

^a WPI = whey protein isolate; CAS = lactic acid casein.

^b Means reported are the least square means for day and treatment $\pm$.01 (the standard error of the mean for Ida Red and Granny Smith data); Scores given to each treatment over time using an hedonic scale of 1-5 with 1=very unacceptable, and 5=very acceptable; n=33 (3 replicates x 11 judges).

^c BF=butter fat, CW=carnauba wax. (Protein:Plasticizer:Lipid)

^{d-f} Columnwise denote significant differences ($p<0.05$).

¹⁻⁴ Rowwise denote significant differences ($p<0.05$) over time.

coated Ida Red apple halves were neither acceptable nor unacceptable until day 2 and day 0, respectively, and were increasingly unacceptable ($p < 0.05$) thereafter.

3.4.6. Correlation of sensory analysis with colorimeter determination of color

3.4.6.1. Colorimeter vs. visual panel results for degree of browning. Regression models were determined between sensory and instrumental evaluation of the degree of browning over time. The data showed a positive correlation with $R^2 = 0.75$ for Ida Red and 0.77 for Granny Smith apple halves. The sensory results for the CAS-based coated Ida Red halves did not correlate with the colorimeter results due to a decrease in degree of browning at the end of the study as reported by the visual panel. Colorimeter and visual panel results followed the same trend for degree of browning for both cultivars on each day of data collection. The WPI-based coated sample including 1.5% BF was always scored as the least brown, WPI-based coated sample with 1.5% CW next, and the CAS-based coated scoring the most brown of the coated samples. With the exception of day 0, the uncoated controls were scored the most brown for every data collection throughout the study.

Large standard deviations occurred within each treatment due to normal color variability, variability in individual apple respiration rate, age of individual apples and slight variation in day to day temperature and RH. A three-way analysis of variance was used to determine the combined effects of the different coatings, days and different panelists on the variability of the results. This was also done to pinpoint major sources of variability and how they might have affected the results. Overall, there was no three-way interaction between coatings, days and panelists. There was, however, a two-way interaction

between panelists and days: panelists ratings varied depending on the day they rated the coatings. This two-way interaction between coating and panelist, however, might have been due to the variability of browning within the coated and uncoated apple halves used in this study. This two-way interaction was difficult to interpret due to the many sources for variability such as individual differences within and between panelists and natural differences in the apple itself.

3.4.6.2. Overall acceptance as it relates to degree of browning. Regression models were performed between degree of browning and visual acceptance of the apple halves as evaluated by the visual panel to determine if the overall acceptance of the coated and uncoated apple halves depended on the degree of browning. The degree of browning was highly correlated to acceptance with $R^2 = 0.98$ for Ida Red; and 0.79 for Granny Smith apple halves. The overall acceptance data followed the same trends as the degree of browning data in section 3.4.7.1.

CONCLUSIONS

1. WPI-based coatings containing 1.5% lipid reduced moisture loss ($p<0.05$) for Granny Smith apple halves, whereas WPI-based coatings containing butterfat reduced moisture loss ($p<0.05$) for Ida Red apple halves stored in LDPE bags.
2. WPI/lipid-based coatings were effective ($p<0.05$) in retarding browning of cut Granny Smith apples for up to 4 days if stored packaged in LDPE bags in refrigerated storage. WPI-based coatings containing 1.5% carnauba wax were effective ($p<0.05$) in retarding browning of cut Ida Red apples for up to 2 days if stored packaged in LDPE bags in refrigerated storage.
3. The CAS-based coatings containing 1.5% carnauba wax retarded moisture loss of both Granny Smith and Ida Red apple halves. However, the CAS-based coatings were not effective in retarding browning of cut Granny Smith or Ida Red apples.
4. Oxygen permeability did not effect the degree of browning cut apples stored in LDPE bags.

WPI/lipid-based coatings could replace preservatives such as lemon juice that impart flavor to the food, to preserve the quality of pre-prepared sliced fruits or vegetables, which are stored in refrigeration until use. The results of this research, however, were found to be cultivar dependent and may not apply to other fruits or vegetables without some modifications in the formula. WPI/lipid coatings could be used on fresh Granny Smith apple slices used at salad bars or used in catering to preserve the quality of minimally processed apples for 2 days if stored in LDPE packaging when stored.

RECOMMENDATIONS

Further work is needed on WPI and CAS-based coatings to improve their barrier properties. This could be accomplished by adding an emulsifier to the WPI coatings containing butterfat, and by developing a more efficient procedure to incorporate a wax lipid into the CAS-based coatings. Using a different lipid, such as acetylated monoglycerides either as an emulsion or bilayer coating may improve the barrier properties of these coatings. Coatings should be prepared in strictly controlled temperature and RH conditions to decrease variability among replications.

Work also needs to be done to improve the performance of these coatings on minimally processed fruits, such as apple halves, as barriers to moisture and color change. This could be accomplished by applying the coating in a more uniform manner or by using an application procedure such as spraying a known quantity of coating evenly across the surface, which would decrease variability within treatments. It is suggested that the total drying time of the coatings should be decreased in half by increasing airflow in the dryer or decrease the amount of solvent used in the coating solution. Drying temperature and RH conditions should be strictly controlled during the application procedure to decrease variability. It is also recommended that future researchers acquire apples that were picked on the same day from the same location to decrease variability in respiration and duration of storage within each apple of each variety.

APPENDICES

APPENDIX 1

Visual Panel Forms

Coated Apple Slices

Visual Acceptance Panel

Monday, February 23, 1998
10:00 a.m. - 12:00 p.m.

and

Thursday, February 26, 1998
1:00 p.m. - 3:00 p.m.

**Take 5-10 minutes to help us develop a
visually appealing, convenient, fresh
sliced apple product and earn a treat for
your time. Just stop by rm. 102 TFS any
time during the above listed times!**

Consent For Visual Panel Members

Department of Food Science and Human Nutrition
Michigan State University

Apple slices coated with an edible coating containing whey protein isolate or lactic acid casein, sorbitol, butter fat or carnauba wax, and water.

You are being asked to participate in this research study, which will determine if edible coatings made with milk proteins have any effect on the color change of apple slices over time. You will be asked to visually determine color differences between coated and uncoated apple slices. There are no foreseeable risks or benefits to you. It will take you 5 minutes to complete each of up to 6 tests (30-40 min. total). The visual panel will take place between July 6, 1998, and July 19, 1998, during which you will evaluate samples at your designated time every 48 hours. You are free to withdraw your consent and to discontinue participation on the panel at any time without penalty. You may contact Dr. Ustunol (project investigator) at 355-0285 or Julie Hazard at 353-9658, if you have any questions regarding this visual panel.

I _____ have read the above list of ingredients and there are none that I find objectionable. I have been informed of the intent and procedures of this experiment and understand the risks involved in participating in this visual panel. I voluntarily agree to participate in the visual panel, which will be conducted on _____, 1998.

Signature

Date

Prescreening Questionnaire

Name _____ Phone (Day) _____
E-mail _____ (Evening) _____

Gender: M or F

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

TIME:

1. Do you plan to be on campus during the summer (6/1/98-6/18/98 for training, and 6/29/98-7/17/98 for panel)? NOTE: I will not be running panel during IFT _____
2. Are there any weekdays that you will not be available on a regular basis? _____
If yes, when? _____
3. Are you willing to come in on a Saturday or Sunday if needed? _____
If yes, which day is better? _____
4. What part of the day are you normally available?
___ Morning (8-11)
___ Early afternoon (11-2)
___ Afternoon (2-5)

HEALTH:

1. Do you have any conditions (i.e., colorblindness) that might impair your ability to determine slight color differences? _____
If YES, explain:
2. Do you take any medications that might affect your visual perception? _____
If YES, explain:

APPLE CONSUMPTION:

1. Do you consume fresh apples? _____
If yes, how often? ___ More than once a week
___ More than once a month
___ Less than once a month

Thank you for your time

Visual Panel Questionnaire

Product: Apple slices

Name: _____ Date: _____

Characteristic: Color

Instructions: Please inspect visually the apple slices provided in the order presented. Evaluate the degree of browning of the cut surface of each apple slice and place an X next to the score that best describes your evaluation of the color. **DO NOT TOUCH OR TASTE SAMPLES.**

Sample	<u>413</u>	<u>220</u>	<u>926</u>	<u>534</u>
1- Not brown (Fresh cut)	_____	_____	_____	_____
2	_____	_____	_____	_____
3	_____	_____	_____	_____
4	_____	_____	_____	_____
5- Moderately brown	_____	_____	_____	_____
6	_____	_____	_____	_____
7	_____	_____	_____	_____
8	_____	_____	_____	_____
9- Very brown	_____	_____	_____	_____

Rank samples in the order of overall visual acceptability: 1=not acceptable; 3= neither acceptable or unacceptable; 5= very acceptable

413 _____
220 _____
926 _____
534 _____

Comments:

APPENDIX 2
Moisture-Loss Data

Table 13. Moisture-loss rate (MLR) of milk protein-based edible coatings on Ida Red and Granny Smith apple halves (@ 4°C, 80% RH).

a)

Moisture-Loss Rate (µg/s) ^{a,b}		
Ida Red		
Treatments ^c	WPI ^f	CAS ^g
1: (7.5:4:0)	0.54 ± 0.14 ^d	1.39 ± 0.26 ^d
2: BF (7.5:2.5:1.5)	0.66 ± 0.13 ^{de}	1.48 ± 0.22 ^d
3: BF (7.5:1:3)	0.90 ± 0.35 ^{de}	1.41 ± 0.26 ^d
4: CW (7.5:2.5:1.5)	0.82 ± 0.32 ^{de}	1.09 ± 0.38 ^d
5: CW (7.5:1:3)	0.84 ± 0.53 ^{de}	1.21 ± 0.56 ^{de}
6: Uncoated control	1.32 ± 0.27 ^e	1.95 ± 0.21 ^e

b)

Moisture-Loss Rate (µg/s) ^{a,b}		
Granny Smith		
Treatments ^c	WPI ^f	CAS ^g
1: (7.5:4:0)	0.47 ± 0.21 ^d	1.47 ± 0.42 ^{de}
2: BF (7.5:2.5:1.5)	0.49 ± 0.22 ^d	1.69 ± 0.48 ^{de}
3: BF (7.5:1:3)	0.54 ± 0.07 ^d	1.59 ± 0.47 ^{de}
4: CW (7.5:2.5:1.5)	0.30 ± 0.07 ^d	1.19 ± 0.13 ^d
5: CW (7.5:1:3)	0.54 ± 0.37 ^d	1.37 ± 0.24 ^d
6: Uncoated control	1.33 ± 0.08 ^e	2.20 ± 0.43 ^e

^a WPI=whey protein isolate; CAS=lactic acid casein.

^b Means ± standard deviation; n=4 for all treatments.

^c BF=butter fat, CW=carnauba wax. (Protein:Plasticizer:Lipid)

^{d-e} columnwise denote significant differences compared to the control.

^{f-g} denote significant differences between protein type in the same apple cultivar (p<0.05).

Table 14. Moisture-loss rate (MLR) of apple halves coated with milk protein-based edible coatings during refrigerated storage (@ 4°C, 80% RH).

Weight-Loss Rate ($\mu\text{g/s}$)^{a,b}		
Treatments^c	Ida Red	Granny Smith
WPI-BF (7.5:2.5:1.5)	0.43 $\pm$ 0.15	0.54 $\pm$ 0.24
WPI-CW (7.5:2.5:1.5)	0.46 $\pm$ 0.14	0.51 $\pm$ 0.26
CAS-BF (7.5:2.5:1.5)	0.50 $\pm$ 0.23	0.61 $\pm$ 0.21
Uncoated control	0.71 $\pm$ 0.21	0.75 $\pm$ 0.13

^a WPI = whey protein isolate; CAS= lactic acid casein.

^b Means $\pm$ standard deviation; n=3 for all treatments. No significant difference was observed.

^c BF=butter fat, CW=carnauba wax; (Protein:Plasticizer:Lipid)

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