Water Vapor Permeability Properties of Edible Whey Protein–Lipid Emulsion Films

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The water vapor permeability (WVP) of whey protein emulsion films was investigated. The exponential effect of relative humidity on the WVP of whey protein films was reduced through lipid incorporation. Film orientation had a significant effect on WVP due to emulsion separation during film formation. Heat denaturation of whey proteins lowered emulsion film WVP. Increasing fatty acid and fatty alcohol chainlengths significantly reduced WVP, as did increasing lipid concentration. The WVPs of fatty acids, fatty alcohols and beeswax were compared in whey protein–lipid emulsion films. Scanning and transmission electron microscopy revealed the crystalline microstructure of lipid particles in emulsion films.

KEY WORDS: Edible film, emulsion film, water vapor permeability, whey protein.

The properties of edible films were recently reviewed (1–3). Edible films can regulate undesirable water vapor transfer by replacing or supplementing synthetic packaging materials with edible films, package waste can be reduced while increasing the recyclability of synthetic packaging materials.

Pure lipid films act as good moisture barriers; however, they often require solvent or high-temperature casting and exhibit poor mechanical properties (4–6). Plasticized whey protein films, on the other hand, can be formed from aqueous solutions at room temperature and exhibit good mechanical properties (7). Whey protein acts as a cohesive structural matrix in multicomponent films. By combining proteins and lipids, the advantages of each may be exploited to form improved film systems.

The properties of composite bilayer films have been studied in the past. Cohesive bilayer films are often difficult to form, and delamination may occur over time. Furthermore, bilayer film formation often requires the use of solvents or high temperatures (4,8), making production more costly and less safe than aqueous emulsion film production. Protein–lipid emulsion film systems can be formed from aqueous solutions and applied to foods at room temperature.

Bilayer film systems composed of hydroxypropyl methylcellulose and various kinds of lipids have been formed by two techniques, both of which use ethanol as a solvent. The “coating technique” involves casting a lipid layer onto a dried edible film, whereas the “emulsion technique” involves adding the lipid to the film-forming solution prior to film casting (9). Hydroxypropyl methylcellulose is not an effective emulsifier; therefore, upon drying, the “emulsion” films separate into bilayer films.

Protein–lipid emulsion coatings were first identified as effective mass transfer barriers by Ukai et al. (9), who patented the use of coatings containing an aqueous water-soluble high polymer and a hydrophobic substance in an aqueous emulsion or suspension for coating food products. This patent targets the use of emulsion coatings on agricultural products. By taking advantage of the good emulsification properties of proteins, film systems in which the lipid is distributed in particles can be formed.

Guilbert (1) investigated emulsified films made from ethanolic solutions of stearic acid, palmitic acid and carnauba wax in aqueous solutions of casein or gelatin. These films formed bilayers during drying, as did the films produced by Kamper and Fennema (8) who used the “emulsion” technique.

Krochta et al. (10) developed emulsion films by combining lipids and caseinate. The addition of lipid increased the water barrier properties of the films. Avena-Bustillos and Krochta (11) continued research on caseinate-based emulsion films. They found that beeswax emulsion films exhibited lower water vapor permeability (WVP) than other lipid emulsion films.

The properties of whey protein–lipid emulsion films have not been previously explored. Therefore, the objective of this study was to examine the WVP properties of whey protein–lipid emulsion films under a variety of relative humidity conditions. High-melting point lipids (fatty acids, fatty alcohols and beeswax) were selected to determine their effects on water barrier properties. The microstructure of these emulsion film systems was examined by scanning and transmission electron microscopy.

EXPERIMENTAL PROCEDURES

Materials. BiPRO whey protein isolate for films was supplied by Le Sueur Isolates (Le Sueur, MN). The lipids incorporated into emulsion films [stearic acid (99+%), palmitic acid (99%), myristic acid (99.5+%), lauric acid (99.5+%), steareryl alcohol (99%), hexadecanol (99%), tetradecanol (97%) and beeswax] were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). Films were plasticized with sorbitol from Fisher Scientific, Inc. (Fair Lawn, NJ). Lithium chloride, magnesium chloride, potassium carbonate, sodium bromide and sodium chloride salts for the formation of saturated salt solutions were purchased from Fisher Scientific, Inc.

Film formation. Aqueous solutions of 10% (w/w) whey protein isolate (WPI) were prepared and heated at 90°C for 30 min in an oil bath (Haake Model No. N4B, Catalog No. 13-874-119C; Fisher Scientific, Inc.) to denature the protein (7). Solutions were cooled to room temperature, and a vacuum was applied to remove dissolved air. Appropriate weights of sorbitol were then added to plasticize the films.

For the formation of emulsion films, whey protein/sorbitol solutions were then reheated to 75°C, and lipid was added. After allowing the lipid to melt, the mixture was homogenized in an Ultra-Turrax T25 homogenizer (Ultra-Turrax, Model T25; IKA-Works, Inc., Cincinnati, OH) for 1 min at 13500 rpm, followed by 4 min at 20500 rpm.

Film casting. Whey protein solutions and emulsions were cast on 14.7-cm (i.d.) rimmed, smooth poly(methylmethacrylate) (Plexiglas) plates located on level granite slabs. In each case, 2,625 g total solids was applied to each plate to minimize film thickness variations. Six films were...
prepared for each film formulation. The solutions and emulsions were spread evenly with a bent glass rod and allowed to dry for approximately 18 h at 40% relative humidity (RH) and 23°C. Dried films could be peeled intact from the casting surface.

**Film thickness measurement.** Thicknesses of the films were measured with a micrometer (Series 436, Catalog No. T436RL-1; L.S. Starrett Co., Athol, MA) after WVP testing to the nearest 0.0001 inches at five random positions around the film. Individual film thickness measurements varied up to 5%. Average values of five thickness measurements per film were used in all WVP calculations.

**WVP determination.** The WVP Correction Method was used for determination of film RH and WVP (12). Plastic desiccating cabinets (Fisher Scientific, Inc., Catalog No. 08-647-28) containing motors (Bodine Motor, Model No. 574, Minarik Electric Co., Fresno, CA) with variable speed controllers (Motor Master, Series 20000; Minarik Electric Co.) and fans (Model No. 607601-01; Refrigeration Supply House, Sacramento, CA) were placed in a 25°C controlled-temperature room. Fan speeds were set to achieve air velocities of 152 m/min in the cabinets. Each cabinet contained a hygrometer (Model No. 605; Airguide, Chicago, IL) to monitor the RH conditions. Prior to each experiment, cabinets were equilibrated to 0% RH with calcium sulfate desiccant (Drierite; Fisher Scientific, Inc.).

Circular test cups were made out of polymethylmethacrylate (Plexiglas). The external base cup dimensions were 8.2 cm in diameter and 1.25 cm in height. The area of the cup mouth was 19.6 cm², and the cup well depth was 1.1 cm. Cup walls were sufficiently thick to render the cup impermeable to water vapor. A film was sealed to the cup base with an 8.2-cm diameter and 0.60-cm tall polymethylmethacrylate (Plexiglas) ring containing a 19.6 cm² opening. Four screws were symmetrically located around the cup circumference. Both sides of the cup contacting the film were coated with silicon sealant (High Vacuum Grease; Dow Corning, Midland, MI).

Deionized water or equivalent amounts of saturated salt solutions were placed in the bottoms of the test cups to expose the film to a high percentage RH inside the test cups. Next, films were mounted in the cups. The distance between the solution and the film was determined both before and after each experiment to the nearest 0.001 inch with a micrometer (Model No. 515; Lufkin Rule Co., Saginaw, MI). Average stagnant air gap heights were used later in WVP calculations.

After assembly, the test cups and mounted films were inserted into the preequilibrated 0% RH desiccator cabinets. Within two hours, steady state had been achieved. Five weights were then taken for each cup at more than 3-h intervals. Four replicates of each film were tested.

Regression analysis of weight loss as a function of time was performed to ensure that a steady-state loss was achieved. Regression coefficients were greater than 0.998. For each experiment, RH at the film's underside and corrected WVP were calculated by the WVP Correction Method, accounting for the effect of the water vapor concentration gradient through the stagnant air layer in the cups (12).

**Statistical analysis.** StatView 4.0 was used for all statistical analyses (Abacus Concepts, Berkeley, CA). Analyses of variance, Fisher protected least significant difference multiple comparisons and regression analyses were performed.

**Scanning electron microscopy (SEM).** Films were fractured under liquid nitrogen and dehydrated under vacuum for three days. Next, films were sectioned and mounted on aluminum stubs with double-stick tape and silver paint. Sputter-coating with gold-palladium alloy was performed at 20 mA. An ISI DS-130 scanning electron microscope was used to examine the microstructure of film cross-sections.

**Transmission electron microscopy (TEM).** Films were fixed, and the protein matrix was stained for 2 h in 2% osmium tetroxide fumes. Ethanol dilutions (15 min each: 1,50%; 1,75%; and 3,100%) followed by propylene glycol dilutions (15 min each: 1,50%; 1,75%; and 3,100%) were used to dehydrate the films. Treated films were then embedded in increasing concentrations of epoxy resin; 8 h, 50%; overnight, 75%; 2 d, 100%. The resin was cured in a vacuum oven and sectioned with a microtome. A Zeiss transmission electron microscope was employed to examine the microstructure of film cross-sections.

**RESULTS AND DISCUSSION**

**Effect of RH.** The exponential effect of RH on the WVP of 50% whey protein/50% sorbitol and 56% whey protein/16% beeswax/28% sorbitol films was examined (Fig. 1). Each point on the graph represents the WVP measured when the underside of the film in the test cup was exposed to the RH shown on the x-axis, with 0% RH at the top-side of the film outside the cup. The exponential nature of this relationship highlights the importance of accurately determining the true RH conditions present when measuring WVP by the WVP Correction Method (12).

The effect of RH on WVP was reduced through the addition of lipid to whey protein emulsion films (Fig. 1), due...