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 in food products, thus improving food quality and extending shelf life. 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 (8). 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+%), stearyl 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 T-25 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 polymethylmethacrylate (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

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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 cm^2, 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 cm^2 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 tetraoxide 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 topside 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

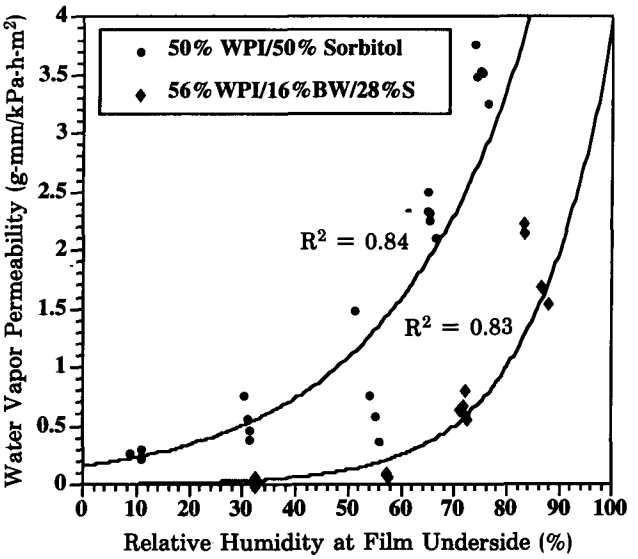

FIG. 1. Effect of relative humidity difference on the water vapor permeability of 50% whey protein isolate (WPI)/50% sorbitol (S) films compared to 56% WPI/16% beeswax (BW)/28% S emulsion films at 25°C. Film top side was at 0% relative humidity.

to the increased hydrophobicity that the lipid imparts to emulsion films. The percent reduction in WVP through the addition of beeswax was greater at high RHs than at low RHs. The addition of lipid to whey protein film systems significantly lowered film WVP in any RH environment.

Hauser and McLaren (13) developed a method to predict hydrophilic film WVPs under any RH gradient by using curves such as those shown in Figure 1. The accuracy of this method was verified by McHugh and Krochta (14) for application to edible films. These curves are extremely useful for food applications by allowing prediction of film permeability properties for food products stored under any RH conditions.

Effect of film orientation. The effect of film orientation in the test cup on WVP of emulsion films was investigated (Tables 1–3). Emulsion separation and creaming during film dehydration are responsible for the nonisotropic nature of emulsion films. After drying, the film side facing the plate was shiny, indicating the presence of increased protein; conversely, the side facing the room was dull. Emulsion instability resulted from the moderate ability of whey proteins as emulsifiers (15). The low density of the lipids was responsible for creaming during film dehydration. Although emulsion separation occurred within these film systems, bilayer films were not formed. Discrete lipid particles remained within the protein matrix, as shown by SEM and TEM.

Emulsion films oriented with the dull, lipid-enriched side facing down (toward the inner, high RH environment of the cup) exhibited, as a whole, significantly lower WVPs than films oriented with the lipid side up. This is due to the hydrophilic nature of whey protein based films and the exponential effect of RH on their WVP properties (Fig. 1). When the lipid side of the film faced the high RH environment, the protein side of the film experienced a lower RH gradient, lowering its contribution to the overall film WVP. These results demonstrate the importance of reporting film orientation along with RH, film thickness and WVP data when examining films containing hydrophilic materials.

Effect of heat denaturation. The addition of increasing concentrations of heat-denatured protein into emulsion films resulted in significantly decreased WVP (Table 1).

TABLE 1

Effect of Heat Treatment on the Water Vapor Permeability of Films of 56% Whey Protein/28% Beeswax/16% Sorbitol Total Solids[a]

Percentage whey protein isolate (WPI) heated in solution	Thickness (mm)	Relative humidity[b] (%)		Water vapor permeability[b] (g-mm/kPa-h-m^2)	
		Down[c]	Up[c]	Down[c]	Up[c]
100% Heated WPI	0.17	94	93	0.86[d]	1.11[e]
75% Heated WPI	0.16	91	89	1.18[e]	1.61[f]
50% Heated WPI	0.17	88	87	1.72[f]	1.95[g]

[a]All values reported are means.
[b]Relative humidity at the inner surface of the film and water vapor permeability (WVP) values were corrected for test cup stagnant air effects by the WVP Correction method (Ref. 12).
[c]"Down" and "Up" refer to the lipid side of the film facing the inside high relative humidity in the cup (Down) vs. the outer 0% relative humidity of the cabinet (Up).
[d-g]Indicate significant differences at $P < 0.05$ from Fisher's protected least significant difference multiple comparison test for comparison of all WVP values.

TABLE 2

Effect of Lipid Chainlength on the Water Vapor Permeability of Films of 56% Whey Protein/28% Beeswax/16% Sorbitol Total Solids[a]

Lipid type	Thickness (mm)	Relative humidity[b] (%)		Water vapor permeability[b] (g-mm/kPa-h-m^2)	
		Down[c]	Up[c]	Down[c]	Up[c]
Fatty alcohols					
Tetradecanol (C14)	0.20	88	87	2.12[d]	2.17[d]
Hexadecanol (C16)	0.18	87	87	2.02[e]	2.11[d]
Stearyl alcohol (C18)	0.15	86	85	1.93[f]	2.05[e]
Fatty acids					
Myristic acid (C14)	0.27	95	95	0.99[g]	0.98[g]
Palmitic acid C16)	0.14	93	92	0.80[h]	0.96[g]

[a]All values reported are means. See Table 1 for abbreviations.
[b]Relative humidity at the inner surface of the film and water vapor permeability values were corrected for test cup stagnant air effects by the WVP Correction method (Ref. 12).
[c]"Down" and "Up" refer to the lipid side of the film facing the inside high relative humidity in the cup (Down) vs. the outer 0% relative humidity of the cabinet (Up).
[d-h]Indicate significant differences at $P < 0.05$ from Fisher's protected least significant difference multiple comparison test for comparison of all WVP values.

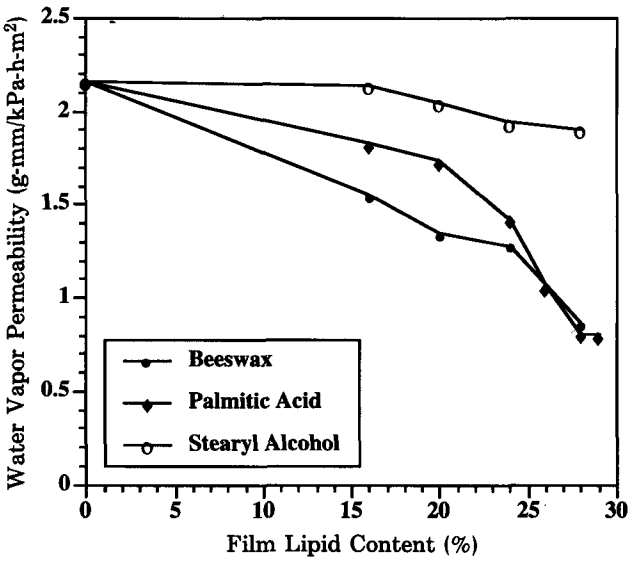

FIG. 2. Effect of lipid concentration on the water vapor permeability of 56% whey protein, x% lipid (beeswax, palmitic acid or stearyl alcohol) and (44-x)% sorbitol emulsion films at 25°C.

At concentrations of less than 50% heat-denatured whey protein, intact films could not be formed, due to insufficient intermolecular bonding within the film. Heat denaturation led to the formation of intermolecular disulfide bonds through thiol-oxidation and thiol-disulfide interchange reactions (7). These reactions have previously been implicated in gelation (16,17) and soy milk film studies (18). Lowered diffusivity and solubility coefficients were responsible for the reduction in WVP in heat-denatured emulsion films. The effect of heat denaturation on the properties of whey protein-based emulsion films had not been previously observed. Heat-induced intermolecular disulfide bonds also resulted in water-insoluble emulsion films. The 100% denatured whey protein was used in all other experiments.

Effect of lipid chainlength. Increasing chainlength of both fatty alcohols and fatty acids resulted in significantly lower WVPs (Table 2). Unfortunately, films could not be cast from lauric acid (C12) or stearic acid (C18) emulsions, due to their extremely high, gel-like viscosity.

Within the same functional category (acid or alcohol), the effect of lipid chainlength on water absorption (solubility) in the lipid was responsible for its effect on film WVP. Solubility results when solute molecules are attracted more strongly to the solvent than to themselves. Water is a highly polar molecule; therefore, the polarity of the lipid affects its ability to absorb water. The lipid polarity is the net result of the number and polarity of the lipid functional groups vs. the extensiveness of the remaining apolar section of the molecule (19). As chainlength increases, the apolar section of the molecule increases in length, resulting in lowered solubility coefficients and WVP values for emulsion films.

Effect of lipid concentration. The effect of lipid concentration on the WVP of palmitic acid, beeswax and stearyl alcohol emulsion films is shown in Figure 2. As lipid concentration increased, film WVP decreased. This effect was more apparent in palmitic acid and beeswax films than in stearyl alcohol films. The downward, convex shape of these curves indicates that the continuous phase in these films was the nonbarrier polymer, and the dispersed phase was the barrier polymer (20).

These curves can be used to tailor film WVP properties to specific food applications. By adding different amounts of lipid, the WVP characteristics of whey protein-emulsion films can be regulated for application to a variety of food products.

Effect of lipid type. Table 3 compares the effect of lipid type (fatty acids, fatty alcohols and beeswax) on the WVP of whey protein emulsion films. Fatty acid emulsion films exhibited very low WVPs, as did beeswax emulsion films. Fatty alcohol emulsion films displayed significantly higher WVP values.

TABLE 3

Effect of Lipid Type on the Water Vapor Permeability of Films of 56% Whey Protein/28% Lipid/16% Sorbitol Total Solids[a]

Lipid type	Thickness (mm)	Relative humidity[b] (%)		Water vapor permeability[b] (g-mm/kPa-h-m²)	
		Down[c]	Up[c]	Down[c]	Up[c]
Palmitic acid	0.14	93	92	0.80[d]	0.96[e]
Myristic acid	0.27	95	95	0.99[e]	0.98[e]
Beeswax	0.17	94	92	0.85[d]	1.24[f]
Stearyl alcohol	0.15	86	85	1.93[g]	2.05[h]
Hexadecanol	0.18	87	87	2.02[h]	2.11[i]
Tetradecanol	0.20	88	87	2.12[i]	2.17[i]

[a]All values reported are means. See Table 1 for abbreviations.
[b]Relative humidity at the inner surface of the film and water vapor permeability values were corrected for test cup stagnant air effects by the WVP Correction method (Ref. 12).
[c]"Down" and "Up" refer to the lipid side of the film facing the inside high relative humidity in the cup (Down) vs. the outer 0% relative humidity of the cabinet (Up).
[d-i]Indicate significant differences at $P < 0.05$ from Fisher's protected least significant difference multiple comparison test for comparison of all WVP values.

The potential of lipid melting point as an indicator for film WVPs was examined. As the melting point increased, the WVP was expected to decrease. The increased intermolecular interactions in high-melting point lipids were expected to lower the solubility coefficients of films. The average WVPs shown in Table 3 and their respective melting points were correlated. The resultant regression coefficient for this linear relationship was 0.7. Therefore, lipid melting point may be used only as a gross indicator of film WVP.

The significant effect of lipid type on WVP can alternatively be related directly to solubility properties. Fatty acids have extremely limited solubility, due to their strong tendency to dimerize through the formation of double hydrogen-bonded networks (19). Beeswax is formed from the association of C24–C36 fatty alcohols and fatty acids to form long ester molecules. The long hydrocarbon chains in beeswax result in its low solubility in most solvents. Fatty alcohols, on the other hand, are soluble in most organic solvents.

Examination of film microstructure. SEM was used to examine the microstructure of 62% whey protein/38% stearyl alcohol emulsion film cross-sections. SEM revealed the presence of crystalline lipid particles within the protein matrix of the emulsion film (Figs. 3 and 4). Figure 4 is a high-magnification micrograph of a stearyl alcohol particle. SEM micrographs of 56% WPI/28% beeswax/16% sorbitol films appeared similar to those shown in Figures 3 and 4, with the beeswax present in discrete crystalline particles. SEM was employed previously to examine the lipid structure in bilayer film systems (4–6). Never before has the structure of protein–lipid emulsion films been examined.

TEM has not been previously employed to examine the microstructure of edible films. Figure 5 is a TEM of a 62% whey protein/38% stearyl alcohol film. This micrograph confirms the presence of discrete crystalline lipid particles in the protein matrix of emulsion films. TEMs of beeswax emulsion films were similar to those of stearyl alcohol films. In future experiments, SEM and TEM will be employed to determine lipid particle sizes and distributions in films to further characterize the relationship between film microstructure and WVP (21).

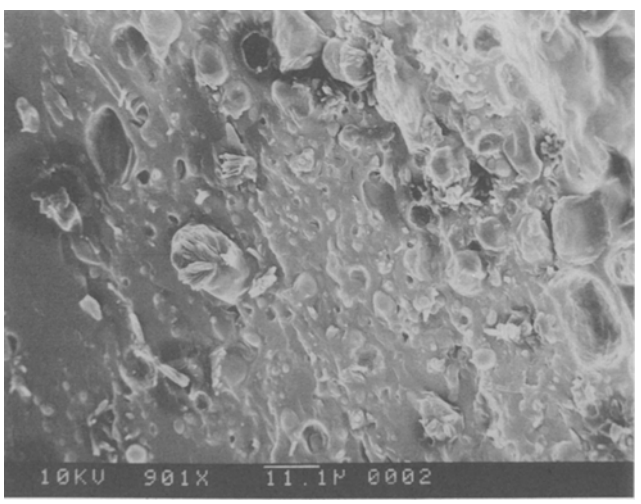

FIG. 3. Scanning electron micrograph of 62% whey protein/38% stearyl alcohol emulsion film cross-section. White bar is 11.1 μm in length.

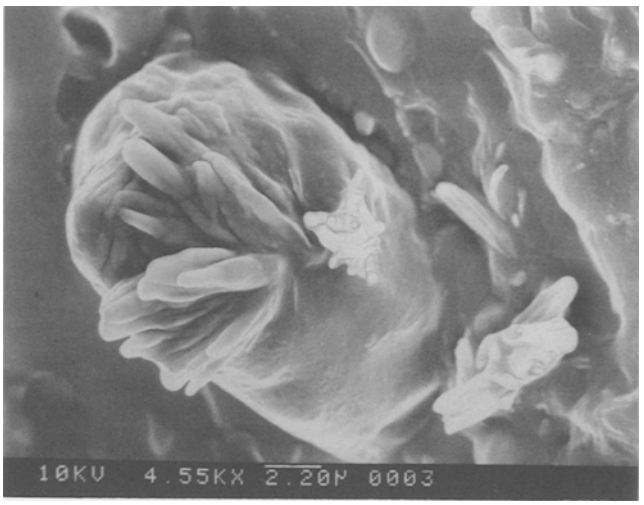

FIG. 4. High-magnification scanning electron micrograph of a lipid particle present in the film cross-section of a 62% whey protein/38% stearyl alcohol emulsion film. White bar is 2.20 μm in length.

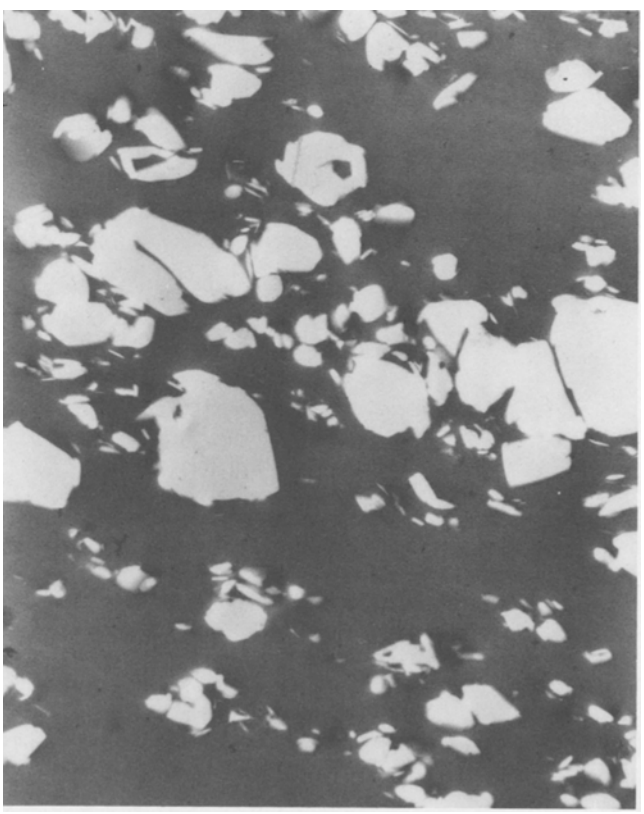

FIG. 5. Transmission electron micrograph of 62% whey protein/38% stearyl alcohol emulsion film cross-section. Dark areas represent the protein matrix stained with osmium tetraoxide and white areas are lipid.

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