Lipid Particle Size Effect on Water Vapor Permeability and Mechanical Properties of Whey Protein/Beeswax Emulsion Films

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Lipid particle size effects on water vapor permeability (WVP) and mechanical properties of whey protein isolate (WPI)/beeswax (BW) emulsion films were investigated. Emulsion films containing 20 and 60% BW (dry basis) and mean lipid particle sizes ranging from 0.5 to 2.0 μm were prepared. BW particle size effects on WVP and mechanical properties were observed only in films containing 60% BW. WVP of these films decreased as lipid particle size decreased. As drying temperature increased, film WVPs decreased significantly. Meanwhile, tensile strength and elongation increased as BW particle size decreased. However, for 20% BW emulsion films, properties were not affected by lipid particle size. Results suggest that increased protein−lipid interactions at the BW particle interfaces, as particle size decreased and resulting interfacial area increased, result in stronger films with lower WVPs. Observing this effect depends on a large lipid content within the protein matrix. At low lipid content, the effect of interactions at the protein−lipid interfaces is not observed, due to the presence of large protein−matrix regions of the film without lipid, which are not influenced by protein−lipid interactions.

Keywords: *Whey protein; beeswax; emulsion films; lipid particle size; water vapor permeability; mechanical properties*

INTRODUCTION

The properties of edible films and coatings have been extensively reviewed by Kester and Fennema (*1*), Guilbert (*2*), Krochta et al. (*3*), Krochta and De Mulder-Johnston (*4*), Debeaufort et al. (*5*), and Baldwin (*6*). In general, edible films and coatings are intended to improve food quality and shelf life by acting as a barrier to moisture, oxygen, flavor, aroma, and/or oil. Their protective function may be enhanced with the addition of antimicrobials, antioxidants, flavors, nutrients, colorants, etc. In addition, edible films or coatings may provide mechanical integrity and improve the handling characteristics of the food. Development has been focused upon barriers containing proteins, polysaccharides, and lipids. Polysaccharides and proteins are good film-forming materials, but they are poor moisture barriers. Lipids, on the other hand, provide a better moisture barrier, but they form brittle films and require solvents or high temperatures for casting (*7*). Addition of hydrophobic lipids to hydrophilic protein- or polysaccharide-based films, by forming an emulsion of the lipid (*8*, *9*) or by laminating the film with a lipid layer (*10*, *11*), greatly improves water vapor barrier properties.

Whey protein isolate (WPI) produces transparent, bland, flexible water-based edible films with excellent oxygen, aroma, and lipid barrier properties at low relative humidity, but WPI films are relatively poor moisture barriers (*8*, *12*, *13*). Incorporation of various lipids into WPI-based edible films greatly improves the water vapor permeability (WVP) of the films (*8*, *14*, *15*). In these films, the lipid is emulsified by whey protein, forming an emulsion film. Factors affecting the WVP of WPI/lipid emulsion films include lipid type and amount (*8*, *14*). Increasing lipid content of films decreases WVP, but the rate at which it decreases depends on the lipid type. McHugh and Krochta (*8*) observed that as the chain length of both fatty acids and fatty alcohols increased, significantly lower WVPs were obtained. Beeswax (BW) and fatty acids were more effective at reducing WVP of WPI-based emulsion films than fatty acid alcohols. These results are consistent with the degree of lipid hydrophobicity.

Previous work by McHugh and Krochta (*16*) indicated that lipid particle size also has an effect on emulsion film WVP. It was found that decreasing mean particle diameter of the film-forming emulsion correlated well with a linear decrease in film WVP. These results were attributed to the immobilization of protein chains at the lipid interface, with resulting formation of a more ordered and tightly cross-linked structure with lower permeability. However, this conclusion was made somewhat tentative due to changing lipid distribution within the film as particle size changed (i.e., more stable emulsions from small lipid particle size versus unstable emulsions from large lipid particle sizes). Mean lipid particle diameter in that study ranged between approximately 0.9 and 2.0 μm, and some lipid phase separation was observed in all of the emulsion films.

Shellhammer and Krochta (*14*) examined the effect of a range of lipid types and amounts on permeability and mechanical properties of stable WPI/lipid emulsion films. The effect of lipid type on WVP correlated with the viscoelastic properties of the lipids (*17*) rather than the WVPs of the pure lipid components, suggesting that the more viscoelastic lipid particles may have deformed to produce an internal interconnecting lipid network. Shellhammer and Krochta (*14*) found a dramatic drop in the WVP of the stable WPI/BW emulsion films at

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Table 1. Homogenization Conditions for WPI/BW Emulsions

desired BW particle size	emulsification technique	20% BW emulsion films	60% BW emulsion films
~0.5 μm	high-shear probe Microfluidizer	2 min at 22000 rpm 4 passes at 6000 psig	2 min at 22000 rpm 4 passes at 6000 psig
~1.0 μm	high-shear probe Microfluidizer	2 min at 22000 rpm 4 passes at 1000 psig	2 min at 22000 rpm 4 passes at 1000 psig
~1.5 μm	high-shear probe mixer	1 min at 15000 rpm 2 min at 22000 rpm	1 min at 15000 rpm 3 min at 22000 rpm
~2.0 μm	hand mixer	1.5 min	2 min

~40% lipid. Beyond this level, further increases in BW content had smaller effects on the final WVP of the films. However, the effect of lipid particle size on film permeability properties at different lipid contents has not been investigated. The Shellhammer and Krochta (*14*) results suggest that the effect of lipid particle size on WVP will depend on lipid content of emulsion films.

Pérez-Gago and Krochta (*18*) observed that drying conditions also have a significant effect on the barrier properties of WPI/lipid emulsion films. As drying temperatures increased, WVP significantly decreased. This was partially attributed to a change in lipid phase distribution within the protein matrix. These films all revealed some emulsion separation during drying. However, the effect of drying conditions on emulsion stability is not clear, because these films possessed large lipid particle size that led to phase separation. For this reason, it would be of great interest to further study the effect of lipid particle size on WVP as the emulsion films are dried at different conditions.

The work reported in this paper was undertaken to further elucidate the effect of particle size in determining the performance of whey protein/lipid emulsion films. We hypothesized that the effect of lipid particle size on the properties of whey protein/lipid emulsion films would be different at low lipid and high lipid contents. We further hypothesized that the effect of lipid particle size would be influenced by film drying temperature.

MATERIALS AND METHODS

Materials. BW (Strahl and Pitsch Inc., West Babylon, NY) was selected as the lipid phase of the WPI emulsion films. BW was emulsified with 10% whey protein solution made from WPI (Davisco Foods International, Le Sueur, MN). Glycerol (Fisher Scientific Inc., Fair Lawn, NJ) was added to all film-forming solutions as a plasticizer.

Film Preparation. Aqueous solutions of 10% (w/w) WPI were prepared and heated for 30 min in a 90 °C water bath to denature the whey protein. The lipid was melted in the hot protein solution, and the plasticizer was added in the amount required to get the final film composition. The protein/plasticizer ratio selected was 3 parts WPI to 1 part glycerol (dry basis), and this ratio was kept constant throughout the study. Emulsion films containing 20 and 60% BW (dry basis) were prepared to study the effect of particle size on the physical properties of the films. Solutions were homogenized to get different particles sizes, ranging from $D(3,2) \approx 0.5$ μm to $D(3,2) \approx 2.0$ μm, which represents the mean diameter over the size distribution or the Sauter mean diameter (19). These particle sizes provided emulsions in the range of stable to unstable. Emulsions with mean particle sizes of approximately 0.5 and 1.0 μm were prepared by first prehomogenizing the sample with a high-shear probe mixer (Ultra-Turrax, model T25, IKA-Works, Inc., Cincinnati, OH) for 2 min at 22000 rpm. This coarse prehomogenization was followed by a fine homogenization with a Microfluidizer HC-5000 (Microfluidics International Corp., Newton, MA), using four passes at 6000 and 1000 psig to obtain approximately 0.5 and 1.0 μm particle size, respectively. Emulsions with mean particle size of ~1.5 μm were prepared with a high-shear probe mixer (Ultra-Turrax, model T25, IKA-Works, Inc.) for 1 min at 15000 rpm, followed by 2 or 3 min at 22000 rpm for 20 and 60% BW, respectively. Last, emulsions containing 20 and 60% BW with mean particle size of ~2.0 μm were homogenized with a hand mixer (Braun MR30, Braun Inc., Lynnfield, MA) for 1.5 and 2 min, respectively. Homogenization conditions are summarized in Table 1. The temperature of the solutions during the homogenization processes was held at 80 ± 3 °C. After homogenization, the emulsions were placed in an ice bath to prevent further denaturation of the whey protein and to crystallize the lipid particles. The film-forming emulsions were degassed at room temperature with a vacuum pump. The films were prepared by pipetting an appropriate volume of the degassed emulsion to maintain a constant mass of solids (3 g of total solids per plate) on a smooth high-density polyethylene (HDPE) casting plate resting on a leveled granite surface. Films were dried at either ambient conditions, which were recorded as 21 ± 1 °C and 55 ± 5% relative humidity (RH), or in an environmental chamber (Tenney-10 model TTUFR-40240, Tenney Engineering, Inc., Union, NJ) at two temperatures (40 and 80°C) and constant relative humidity (40% RH). The chamber was equipped with a one-setting fan that circulated interior air at ~20 m/min. Drying times at these three drying conditions were 20 h, 4 h and 25 min, and 2 h and 33 min, respectively. Drying temperatures were chosen above and below the melting point of the BW, which is 62−64 °C (*20*), so that the lipid stayed in the liquid or crystalline state during drying.

Lipid Particle Size Analysis. McHugh and Krochta (*16*) confirmed that lipid particle sizes in films measured by scanning electron microscopy were similar to those in emulsions. Thus, laser light scattering was used to examine the particle sizes of the emulsions prior to film formation, and results were assumed to be similar for the dried emulsion films. Lipid particle size frequency of the film-forming emulsions was determined using a Malvern Mastersizer (model MS 20, Malvern Instruments Ltd., Malvern, U.K.). Three measurements of each emulsion were made to ensure no errors during measurements, and the results were averaged. The relative refractive index of the lipid phase to water was set at 1.08, and the absorption was set at 1.0. A 45 mm focal length and a 2.2 mm beam length were used. The obscuration in all of the measurements was 0.15 ± 0.02. The mean particle size was recorded as the $D(3,2)$ diameter.

WVP Measurements. A modification of the ASTM E96-95 (*21*) gravimetric method for measuring WVP was employed (*22*). Six films were cast for each emulsion formulation, each film from newly prepared film-forming emulsion, which corresponded to different replicates. Upon drying, three to six films were chosen for WVP measurements on the basis of lack of defects, such as cracks or pinholes. From each film, two samples were cut and mounted on Plexiglas test cups containing 6 mL of water, with the film surface that had been exposed to air during drying facing either the low RH environment ("facing up") or the high RH environment ("facing down"), allowing detection of any phase separation within the film. The cups were placed in desiccator cabinets containing fans and held at 0% RH using anhydrous calcium sulfate (W. A.

Table 2. Average Lipid Particle Sizes in WPI/BW Emulsions

	mean particle diameter of 20% BW emulsions (μm)	mean particle diameter of 60% BW emulsions (μm)
Microfluidizer (6000 psig)	0.51 ± 0.05	0.54 ± 0.02
Microfluidizer (1000 psig)	1.03 ± 0.12	1.01 ± 0.15
high-shear probe mixer	1.51 ± 0.04	1.82 ± 0.12
hand mixer	1.84 ± 0.12	2.28 ± 0.10

Hammond Drierite Co., Xenia, OH). Weights taken periodically after steady state was achieved were used to calculate the percent RH at the film underside and the resulting WVP.

Film Thickness Measurements. Film thickness was measured after the WVP test using a caliper micrometer (No. 7326, Mitutoyo Mfg. Co. Ltd., Japan), taking measurements at six random positions of the film. Averaged values of the six thickness measurements per film were used in all of the WVP calculations. The thickness for individual films ranged from 0.13 to 0.20 mm (5.0−8.0 mil).

Tensile Properties Determination. Film solutions were cast onto rectangular 23 cm × 30 cm, rimmed, smooth HDPE plates, by applying 10.8 g of total solids/plate to minimize thickness variations among treatments. After drying at 40 °C and 40% RH, the films were conditioned at 100% RH for <1 day. This preconditioning enabled ease of handling and cutting of the films. Test pieces of film were cut using a striking die (The Right Image, Sacramento, CA). The cut film samples had a rectangular center section measuring 15 mm wide by 100 mm long, flaring to 25 mm by 35 mm square section on each end, which provided a greater grip area. Before testing, all film strips were equilibrated for 48 h at 53% RH in a cabinet using magnesium nitrate (Fisher Scientific Inc.) saturated solution at room temperature (23 ± 2 °C).

Tensile measurements [tensile strength (TS), Young's modulus (YM), and percent elongation at break (%E)] were performed following the procedure outlined in ASTM method D882-97 (*23*). The ends of the equilibrated film strips were mounted and clamped with pneumatic grips on a Universal Testing Machine (model 1122, Instron Corp., Canton, MA) with a 500 kg load cell. The initial gauge length was set to 115 mm, and films were stretched using a crosshead speed of 50 mm/min. Testing conditions were controlled throughout the measurements and held constant at 50 ± 5% RH and 23 ± 2 °C. Tensile properties were calculated from the plot of stress (tensile force/initial cross-sectional area) versus strain (extension as a fraction of the original length), using Series IX Automated Materials Testing System software (Instron). Mechanical properties reported are maximum tensile stress (MPa), elongation at break (%) and elastic modulus (MPa).

Statistical Analysis. The effects of lipid particle size, lipid amount, and drying conditions on the final film WVP and mechanical properties were determined by using a split-plot design with drying conditions being the blocked variable, lipid particle size the main treatment, and lipid amount the subtreatment. Statistical analysis was performed using SAS software (*24*). Duncan's multiple-comparison test ($p \leq 0.05$) was used to determine significance of differences between means in the cases that were significantly different.

RESULTS AND DISCUSSION

Lipid Particle Size Analysis. The mean particle diameters of the emulsions containing 20 and 60% BW are shown in Table 2. The 60% BW emulsions homogenized with the high-shear probe and the hand mixer had larger mean particle diameter than emulsions containing 20% BW (Table 1), even though homogenization times were longer. However, emulsions prepared using the microfluidizer for similar homogenization conditions had similar particle sizes independent of the BW content. This could be due to the difference in emulsification methods. To produce an emulsion, it is usually necessary to put a considerable amount of mechanical energy into the system. Average droplet size is a function of the energy input (*25*). Among the techniques used for emulsification, the high-shear probe and hand mixer provide the lower energy input. Thus, an increase in lipid content would more likely affect the final particle distribution of the emulsions.

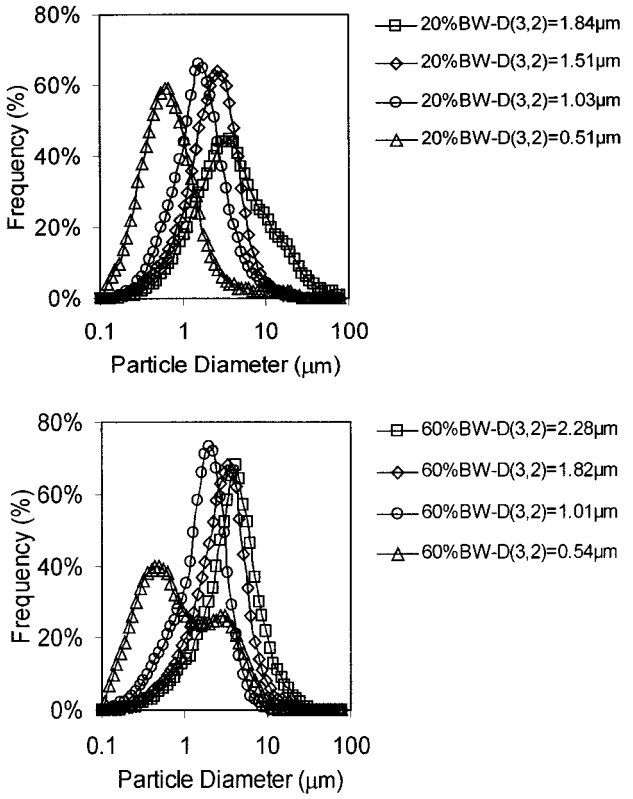

Figure 1. Particle size frequency of WPI/BW emulsions containing 20 and 60% BW.

In emulsion systems, the particle size of the lipid droplets is usually reported as the mean particle value. This value is the diameter of a hypothetical particle that represents the total number of particles in the sample (*26*). However, emulsification gives a distribution of lipid particle sizes, with lipid droplets having larger and smaller particle sizes than the mean particle value. To more easily interpret results, values are given as mean particle size diameter. However, one must consider the particle size distribution of the emulsion when making conclusions about particle size effect on the barrier properties of the resulting emulsion films. WVPs of the emulsion films are the result of a range of lipid particle sizes. Figure 1 shows the particle size frequency of the different WPI/BW film-forming emulsions. All emulsions were normally distributed except for 60% BW emulsions, which presented a bimodal distribution. At this small average particle size (i.e., large surface area) and lipid content, there might not have been enough whey protein available to bring all of the particles to the smaller particle size represented by the left peak of the bimodal distribution. Overall, the particle size frequencies of emulsions with intermediate mean particle size [i.e., $D(3,2) \approx 1.0$ and 1.5 μm] had significant overlap. However, from Figure 1 we can say that emulsions with mean particle sizes around 0.5 and 2.0 μm had different size frequencies with less overlapping, even though 60% BW emulsions with mean particle size ~0.5 μm had a small percentage of lipid particles with larger values.

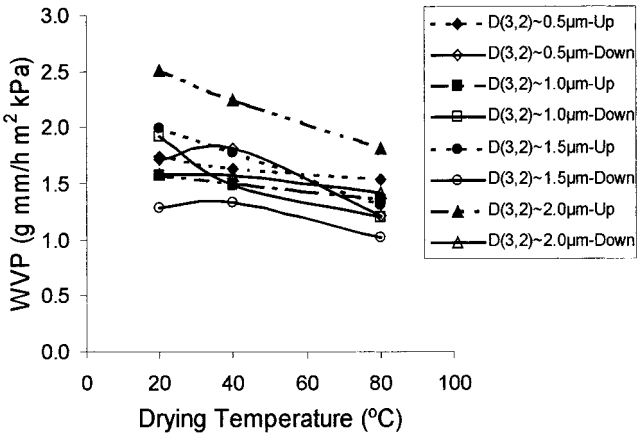

Figure 2. Effect of drying conditions on WVP of WPI/glycerol/BW (60:20:20) emulsion films. Each point represents the average of at least three replicates. Standard deviations ranged from 0.07 to 0.40.

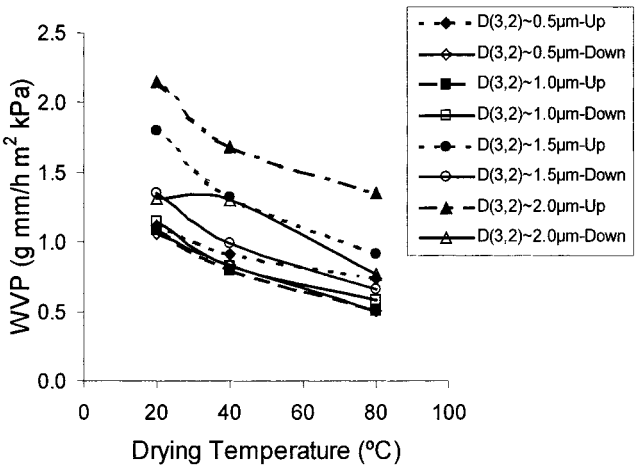

Figure 3. Effect of drying conditions on WVP of WPI/glycerol/BW (30:10:60) emulsion films. Each point represents the average of at least three replicates. Standard deviations ranged from 0.06 to 0.30.

Effect of Lipid Particle Size and Drying Temperature on Film Appearance. Lipid particle size, lipid content, and drying conditions affected the final appearance of the films. Whey protein films are transparent in nature; however, when lipids are added, the films lose transparency. The degree of opacity depends on the lipid content and particle size. In general, films with small particle size and low lipid content were translucent; however, as particle size and lipid content increased, the films became more opaque. Lipid particle size also affected lipid distribution within the film. Those films with larger particle size showed a lipid-enriched layer as the film dried, which correlated with WVP results. Differences in film appearance were also found to depend on film drying conditions. Films dried at 80 °C and 40% RH presented some areas with aggregation of lipid droplets. These films also presented some lipid migration toward the edges. This could be attributed to the fact that because the films started drying from the edges of the casting plates, the lipid phase tended to migrate toward this more hydrophobic (lower moisture) area. However, only samples with uniform lipid distribution and lack of defects were obtained to measure WVP.

Effect of Drying Temperature on WVP and Emulsion Stability. Figures 2 and 3 show the effect drying conditions had on WVP of the 20 and 60% BW emulsion films, respectively. Analysis of variance confirmed that drying conditions had significant effect on WVP ($p < 0.05$). Results show that increasing the film drying temperature produced films with decreased WVP. Similar results were also reported by Pérez-Gago and Krochta (*18*) for 10% WPI and WPI/lipid emulsion films. These results were attributed to a better polymer arrangement and cohesion within the film polymer matrix as drying temperature increased. Furthermore, drying of the emulsions at temperature above or below the lipid melting point could modify the lipid crystalline morphology and/or lipid distribution within the matrix, giving films with different permeabilities.

Lipid phase separation within the film was determined by measuring WVP as a function of film orientation on the WVP test cup. Figure 4 shows the effect of particle size and drying temperature on WVP and lipid phase separation. Lipid phase separation occurred in all films having mean lipid particle diameters of approximately 1.5 and 2 μm. However, no phase separation effect was observed at lower mean particle sizes. This can be explained by lipid particle size effect on emulsion stability. Because the creaming rate depends on the square of lipid droplet diameter according to Stoke's law, large droplets suspended in the solution will cream more rapidly (*25*). Interestingly, an increase in drying temperature did not modify creaming behavior. Phase separation was not influenced by drying temperature for any of the particle diameters, even though a decrease in solution viscosity as temperature was increased could have induced creaming of the small lipid droplets or enhanced creaming of the larger droplets. These results suggest that lipid particle size has a greater effect than film drying temperature (i.e., solution viscosity) in producing lipid phase separation within the protein matrix.

Effect of Film Orientation and Lipid Particle Size and Content on WVP. The effect of film orientation, lipid particle size, and content on WVP are shown in Figure 4. In general, WVP results present some variability. As previously mentioned, lipid particle sizes of the emulsions are reported as the diameter of a hypothetical particle, which represents the total number of particles in the sample. This could be the reason for some of the result variability because WVP values are attributed to a single lipid particle size. The ideal, to fully understand lipid particle size effect on emulsion film permeability, would be to obtain films with a single lipid particle size or at least with narrower particle size distributions sufficiently different from each other. From Figure 1, it can be seen that emulsions having mean particle sizes around 0.5 and 2.0 μm have different distributions with little overlapping, whereas intermediate distributions have considerable particle size overlapping. Nevertheless, some significant differences in WVP were observed for the emulsion films, and conclusions were made when possible.

As expected, films containing higher BW content had lower WVP than films with lower BW content ($p < 0.01$). Analysis of variance showed no particle size effect for WPI/BW films containing low (20%) BW content or when the enriched BW layer of WPI/BW films containing high (60%) BW content was exposed to the high RH side during WVP measurements. However, analysis of variance showed that film WVP decreased as emulsion particle size decreased for films containing 60% BW,

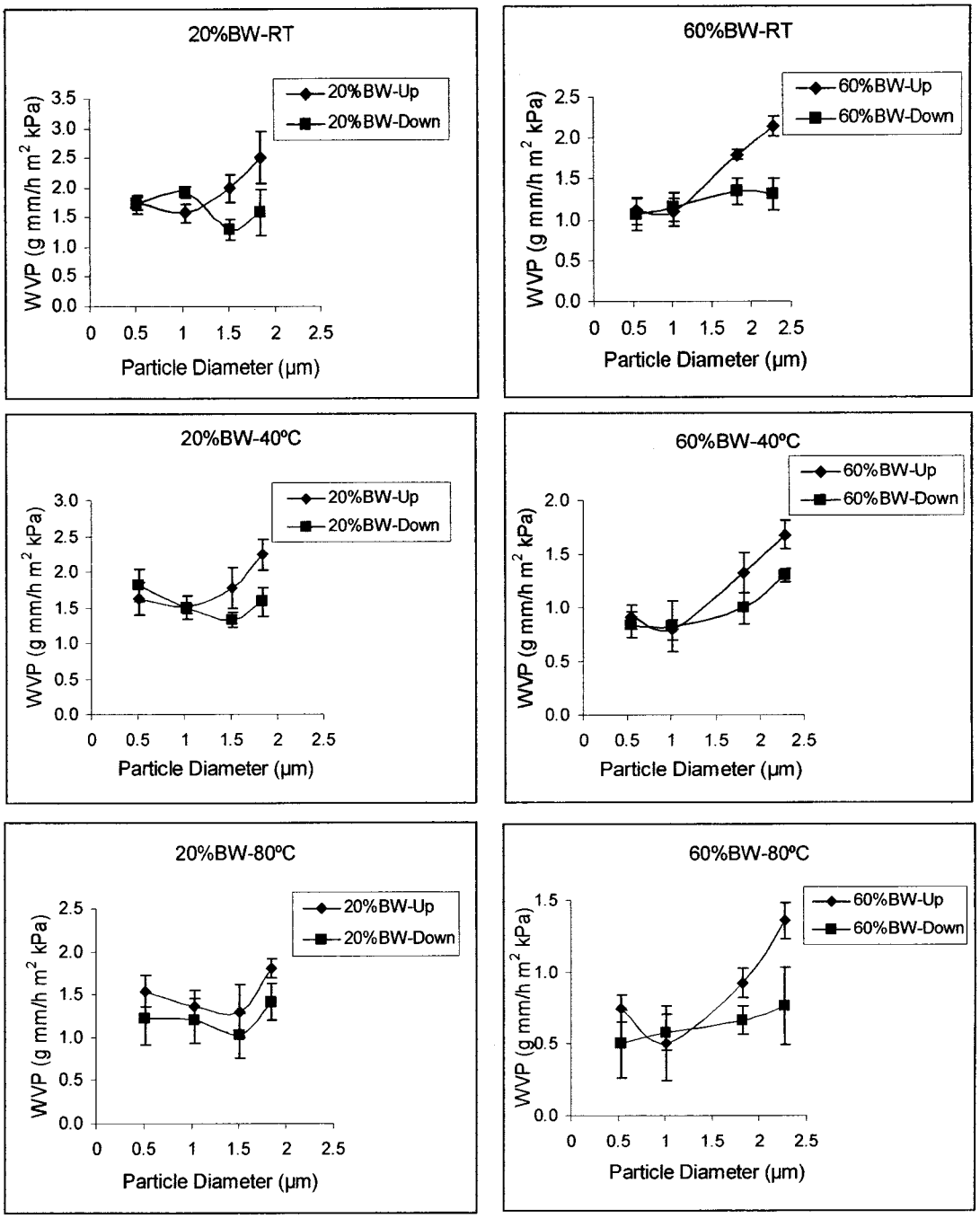

Figure 4. Effect of lipid particle size on WVP of WPI/BW emulsion films dried at different drying temperatures. Each point represents the average of at least three replicates. Error bars indicate standard deviations. RT, room temperature.

when the enriched BW layer faced the low RH side during WVP measurements.

The particle size effect on WVP only at high lipid content could be explained by the fact that at low lipid content the hydrophilic protein matrix still dominates in the final film composition. Therefore, any increase in the immobilization of protein chains at the lipid interface with resulting formation of a more ordered and tightly cross-linked structure, as lipid particle size is decreased, probably does not influence significantly the final barrier properties of films with low lipid content. However, as lipid content increased, the extent of protein immobilization at the protein−lipid interface becomes more important, and films presented lower permeability as surface area increased. This effect is more easily seen when the enriched lipid layer is facing the low RH side during WVP measurements. When there is phase separation in WPI/BW emulsion films and the enriched lipid layer film is exposed to the high RH side during WVP measurements, the enriched lipid layer offers a greater barrier to water vapor than when the more hydrophilic phase is facing the high RH side. This greater barrier could overwhelm any lipid particle size effect on reducing the WVP of the more hydrophilic, protein-rich phase. However, this effect is seen when the more hydrophilic, protein-rich phase is exposed to the high RH side during WVP measurement.

McHugh and Krochta (*16*) studied the effect of particle size on WVP of WPI/BW emulsion films with 28% BW. After examining the relationship between mean particle diameter (ranging between 0.9 and 2.0 μm) and the averaged WVP (values obtained using up and down

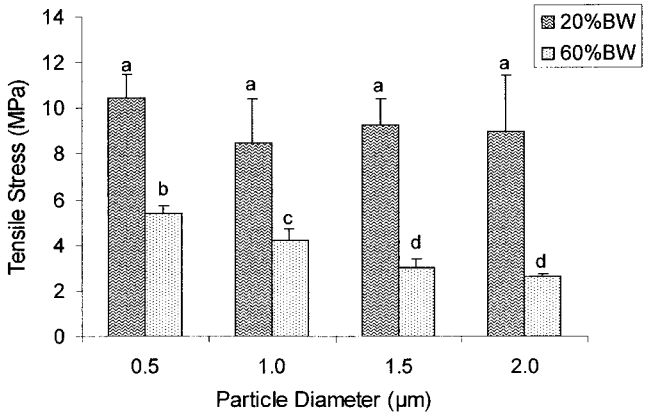

Figure 5. Effect of lipid particle size on tensile strength of WPI/BW emulsion films. Error bars indicate standard deviations. Means with different letters are significantly different at $p = 0.05$.

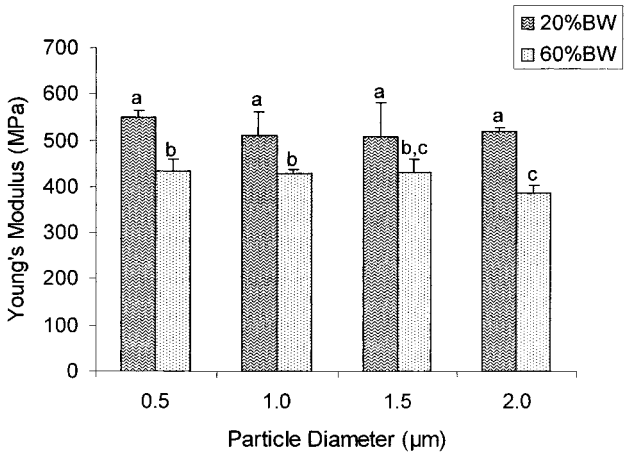

Figure 6. Effect of lipid particle size on Young's modulus of WPI/BW emulsion films. Error bars indicate standard deviations. Means with different letters are significantly different at $p = 0.05$.

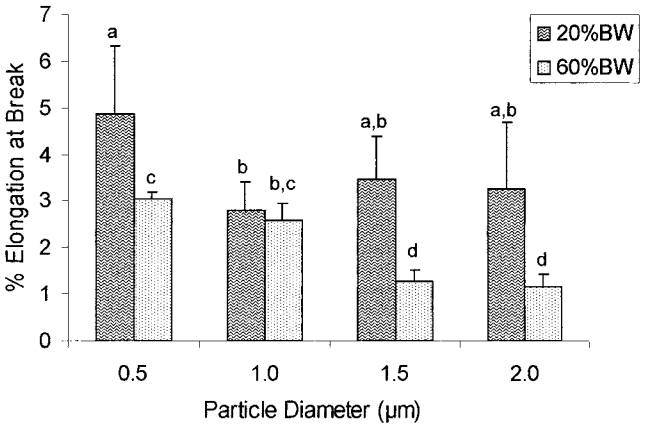

Figure 7. Effect of lipid particle size on elongation at break of WPI/BW emulsion films. Error bars indicate standard deviations. Means with different letters are significantly different at $p = 0.05$.

orientations during permeability measurements), they found that decreasing mean emulsion particle diameter correlated well with a linear decrease in film WVPs. In the present work, lipid particle size effect on WVP depended on the BW content and on film orientation during WVP measurements. For 60% BW emulsion films facing up, WVP shows an initial linear decrease with mean particle diameter, leveling off at low BW mean particle diameter. This could suggest that further decrease in lipid particle size distribution does not further improve permeability properties through the WPI/BW emulsion films. However, the existence of a bimodal distribution may have caused the deviation in the trends for 60% BW emulsion films shown in Figure 4. If the distribution would have been unimodal around $D(3,2) = 0.5\,\mu$m, there may have been a further decrease in WVP. Due to the overlapping in lipid particle size it would be of interest to prepare emulsions with narrower and lower particle size distributions to completely elucidate particle size effect on WVP.

The results shown in Figure 4 for 60% BW suggest that films formed from stable emulsions had lower WVP than films from unstable emulsions. Kamper and Fennema (*27*) found that when a pure lipid is laminating a hydrophilic film, forming a continuous bilayer, the barrier against water vapor transfer was higher than when a stable emulsion film was formed. However, it is impossible to form a continuous bilayer film from an emulsion made with whey protein, due to the emulsifier character of the proteins. Figure 4 shows that producing a film from an unstable emulsion which shows some phase separation does not give a better film barrier compared to a film from a stable emulsion. We hypothesize that decreasing lipid particle size within emulsion films improves the final barrier properties of the films, because of an increase in protein immobilization due to larger lipid−protein interfacial area.

Effect of Lipid Particle Size on Mechanical Properties. Previous results showed that drying conditions had no effect on mechanical properties of WPI/BW emulsion films (*18*). For this reason, films for mechanical property determination were dried only at 40 °C and 40% RH. Mechanical properties of emulsion films containing 20 and 60% BW were compared as related to lipid particle size (Figures 5−7). As expected, films with lower BW content were stronger, stiffer, and more extendible than films with high BW content, because increasing the lipid level reduces the strength and flexibility of the protein phase ($p < 0.01$). Consistent with no effect on WVP, BW particle size had no effect on TS, YM, and %E for films containing 20% BW. However, consistent with the effect on WVP, BW particle size had an effect on TS and %E for WPI/BW films containing 60% BW. For these films, both properties significantly increased as BW particle size decreased. The increase in strength as particle size decreased for the 60% BW emulsion films could be due to an increase in protein immobilization at the BW particle interface as BW particle size decreases. The increase in protein immobilization at the BW particle interface could also increase film elongation before break. However, changes in lipid particle size did not influence mechanical properties for films with low (20%) lipid content.

Conclusion. The effect of lipid particle size on WVP and mechanical properties has been shown to depend on lipid content and film orientation during WVP measurements. As lipid content increased, a decrease in lipid particle size improved the WVP of the WPI/BW emulsion films, probably due to an increase in protein immobilization at the lipid−protein interface as lipid content became more important in the film. This effect seems to be supported by the increase in film tensile strength. For those films that showed lipid phase separation during drying, WVP was not affected by lipid

particle size when the enriched lipid phase was facing the high RH side. This could be attributed to the greater physical barrier that the water vapor experienced when the enriched lipid phase was exposed to the high RH side during the WVP measurement, making any effect due to lipid particle size overwhelmed by this factor.

The use of emulsification techniques that provide narrower lipid particle size distributions with little or no overlapping is required to better understand lipid particle size effects on the barrier and mechanical properties of protein emulsion films.

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