Effect of Fatty Acid Type on Dispersed Phase Particle Size Distributions in Emulsion Edible Films†

Craig P. Sherwin, David E. Smith,* and R. Gary Fulcher

Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Avenue, St. Paul, Minnesota 55108

Edible films used as moisture barriers can enhance food quality. Dispersed phase particle size distributions of microfluidized whey protein−fatty acid emulsion films were measured to determine the effect of fatty acid type on microstructure. Emulsions of saturated fatty acids from C_{14} to C_{22} and whey protein isolate were prepared by microfluidization and analyzed by polarized light microscopy coupled with digital image analysis. Particle size increased with increasing chain length, and at least two populations of fatty acid particles were observed. Chain length effect on particle size may be used to explain functional property differences in future experiments.

Keywords: *Milk proteins; edible film; microscopy; emulsion; digital analysis*

INTRODUCTION

Edible films can greatly enhance the stability and quality of foods by functioning as barriers to gases, moisture, lipids, and aromas; as strong biodegradable packages; or as carriers of additives (Krochta and De Mulder-Johnston, 1997; Koelsch, 1994; Chen, 1995). Whey protein films provide excellent strength and sensory properties but are relatively poor moisture barriers (McHugh et al., 1994; McHugh and Krochta, 1994a,b; Chen, 1995). The incorporation of lipids into whey protein films greatly improves their water vapor barrier properties (Chen et al., 1993; McHugh and Krochta, 1994a,b; Stuchell and Krochta, 1995; R. Banerjee and H. Chen, personal communication, 1996; Fairley et al., 1997; Shellhammer and Krochta, 1997). Lipid can be incorporated into a protein or carbohydrate film formulation either by the formation of a bilayer film with a lipid applied as a laminate over the base film or as an emulsion. A tendency for bilayer films to delaminate over time, develop pinholes or cracks, exhibit poor strength, and exhibit nonuniform surface and cohesion characteristics has been reported (Kamper and Fennema, 1984; Greener and Fennema, 1989). However, bilayer films have shown greater barrier and mechanical properties than emulsion films (Park et al., 1996; Debeaufort et al., 1993; Callegarin et al., 1997).

An emulsion of relatively impermeable lipid within a continuous matrix sets up a more tortuous path, or increased path length, for diffusing molecules than does a pure hydrophilic matrix (Koelsch, 1994). Particle size in the dispersed lipid phase is thought to govern this "tortuosity" of the film matrix or the degree to which the matrix lengthens the permeant path (McHugh and Krochta, 1994a). Additionally, the presence of multiple dispersed particles increases the potential for interaction between the dispersed and continuous phases (Sherwin, 1998). Dispersed particles of smaller diameter should result in a more ordered and tightly cross-linked structure (Banerjee and Chen, personal communication, 1996). This could mean a stronger, less porous, and more rigid film. A more even distribution of the lipid would affect film properties in the same way by eliminating areas of low lipid concentration to which permeating water would be attracted. Therefore, a fine particle size and an even distribution of lipid are important to the effectiveness of an emulsion film (Debeaufort and Voilley, 1995), and homogenization becomes a significant factor.

Microfluidization is a recently patented technique that utilizes the shearing forces of ultrahigh-pressure homogenization as well as the severe stress of head-on collision to create a finer particle size emulsion than other means of homogenization. Microfluidization improves the moisture barrier properties and tensile strengths of WP edible films (Chen et al., 1993; Banerjee and Chen, personal communication, 1996).

Quantitative information on the microstructure of edible films is scarce. Particle size in the dispersed phase, measured by laser light scattering, is the most frequently studied attribute (McHugh and Krochta, 1994a; Deabeaufort and Voilley, 1995; Fairley et al., 1997; Shellhammer and Krochta, 1997). Because samples must be in a liquid form for this instrument, measurements are often taken on the undried emulsion rather than the dried and set film (McHugh and Krochta, 1994a; Fairley et al., 1997; Shellhammer and Krochta, 1997) or by dissolving the film in water and measuring the remaining solid lipid particles. Light microscopy digital image analysis (DIA), while not previously used on edible films, has been widely used on cereals to measure starch granule and other particle size distributions (Harrigan, 1997). This technique can be useful for the collection of data directly from a dried and set film.

Electron microscopy (EM) has frequently been used to examine the microstructure of edible films and liquid emulsions (Gejl-Hansen and Flink, 1977; deMan, 1982; Kamper and Fennema, 1984; Greener and Fennema,

* Author to whom correspondence should be addressed [telephone (612) 624-3260; fax (612) 625-5272; e-mail dsmith@che2.che.umn.edu].

† Published as Paper 98-1-18-0006 of the contribution series of the Minnesota Agricultural Experiment Station based on research conducted under Project 18-24 and supported by the National Dairy and Promotion Board.

10.1021/jf9802981 CCC: $15.00
Published on Web 10/02/1998

1989; Kester and Fennema, 1989; Wong et al., 1992; Debeaufort et al., 1993; Rosenberg and Lee, 1993; McHugh et al., 1994; McHugh and Krochta, 1994a,b; Banerjee and Chen, 1995; Park et al., 1996). In general, the observed physical differences do not relate well to chemical phenomena. Quantitative EM is difficult because the means to distinguish phases without disturbing microstructure are limited. Furthermore, preparation of samples is very labor-intensive and requires the use of extreme temperatures or chemical treatments.

Whey protein−beeswax emulsion films produced under different degrees of high-shear mixing homogenization yielded water vapor permeabilities (WVP) that correlated with increased particle size (McHugh and Krochta, 1994a). However, particle size was unable to account for the WVP of films containing different types of lipid (Shellhammer and Krochta, 1997). The effect of fatty acid (FA) chain length on WVP of certain emulsion films has been previously reported (Kamper and Fennema, 1984; McHugh and Krochta, 1994a,b; Fairley et al., 1997; Shellhammer and Krochta, 1997). Accurate particle size data are important in the understanding of the microstructure of edible emulsion films and in the development of mathematical models describing the relationship between dispersed lipid and WVP. The objective of this research was to measure the effect of fatty acid type on particle sizes of microfluidized whey protein isolate (WPI)−FA emulsion films.

MATERIALS AND METHODS

Emulsion Preparation. Six FA treatments were formulated for the samples: myristic, palmitic, stearic, arachidic, and behenic acid (Sigma Chemical Co., Fair Lawn, NJ), representing 14−22-carbon chain lengths plus a reference containing no FA. A stock solution of 10% (w/v) WPI powder (BiPro lot LE-204-5-0-420; Davisco International, Le Sueur, MN; 96.2% protein) was prepared and deaerated in a sonicator (Branson Ultrasonics Corp., Danbury, CT) for 30 min or until no visible bubbles were present. The degassed solution was held in a water bath at 90 °C for 30 min. FA was melted on a heated stir plate while sorbitol was added. A 100 mL aliquot of denatured WPI (dWPI) at ~90 °C was added to the melted FA−sorbitol mixture to give concentrations of 15.81% FA, 35.23% sorbitol, and 48.95% dWPI on a dry weight basis. The mixture was immediately microfluidized (Microfluidics Corp., Newton, MA) at 7500 psi (51.7 MPa) with the sample making two passes through the machine. Each run was randomly ordered. Emulsions were passed through an ice water bath immediately following microfluidization. Exit temperature was 30 °C, below the melting points of the lipids studied.

Specimen Preparation. Microscope slides were dipped into the five emulsion formulations plus the reference and allowed to drip from one end until a thin layer formed. Films were set at 35 °C for 4 h in an incubator (VWR, South Plainfield, NJ) modified to circulate and vent air. Dried films were stored at 23 °C and 0% relative humidity to prevent mold growth.

Microscopy and DIA. EM was performed to determine the porosity of the reference film and the relative homogeneity of emulsion films. Cross sections were fractured under liquid nitrogen, sputter-coated with Pt (1.0 nm), and examined in a cold-field emission "in-lens" type low-voltage SEM at 300−100000× (S900, Hitachi Scientific Instruments, Inc., San Jose, CA).

For light microscopy, a Zeiss Universal microscope (Carl Zeiss, Eching, Germany) fitted with polarizing filters and a Neofluar 10× objective lens was used. Images of fat crystals were captured with a charged couple device video camera module (model XC-77; Sony Co., Ltd., Tokyo, Japan) and were sent to an IBAS digital image analysis system (Konstron Elektronik, Zurich, Switzerland). The conversion to a high-contrast image by IBAS was custom developed to produce an accurate representation of the dispersed phase particles. Images were processed in the following steps: emphasizing, multiplying, discriminating, identifying frame and objects, and measuring.

For each specimen, areas of at least 3000 dispersed particles were measured. Because particle size was not normally distributed, ANOVA could not be performed on the data directly. Data were sorted in ascending order and comparisons were made at the 50th (median), 75th, 90th, 95th, and 98th percentiles (Suttherawattananonda et al., 1997). For example, the 95th percentile would represent a particle area that is larger than 95% of all of the other particles. Data were analyzed by the MacAnova statistical analysis program (Oehlert & Bingham, Minneapolis, MN). A multiple comparison test (Tukey's) was employed to determine statistical differences among formulations. Five analyses were performed (one for each percentile) for five sets of values (five replications of film preparation). Two of the replications were prepared with duplicate films to determine a measurement error value according to the ANOVA model:

$$\text{particle size} = \text{formulation} + \text{duplication} + \text{replication} + (\text{form} \times \text{rep}) + \text{error} \quad (1)$$

To obtain P values for the same parameters in eq 1, a second ANOVA was performed using data from all five replications. The measurement error value from the first ANOVA was used in the Tukey multiple comparison test due to its increased sensitivity.

RESULTS AND DISCUSSION

Specimen Preparation. Preliminary specimen preparation trials were performed using chemical fixatives and dyes to obtain films that were stable and showed contrast between lipid and protein. However, a set and dried film can be considered inherently fixed if kept at low humidities. The highly disulfide cross-linked polymerized WP matrix should require no additional fixation procedures. This inherent film stability can eliminate the need for sample preparation procedures that require hydration and chemical fixatives. Polarizing microscopy can differentiate phases without the use of stains. This was determined to be the most effective method of analysis, to simplify the preparation procedure without sacrificing contrast in the images.

Preliminary Microscopy. SEM showed that the reference film was free of air bubbles and blemishes and contained no resolvable microstructure. Emulsions were very homogeneous over the entire cross section. Polarized light microscopy confirmed the low level of defects in the films. At 10, 40, and 100×, the pure protein film showed no structure above the resolution of the microscope. A homogeneous protein sheet was observed, with apparent sorbitol crystals, bubbles, or foreign matter embedded at an approximate frequency of only 1 object per 10 fields of view. Nonlipid anisotropic materials can include undissolved sorbitol, aberrations, and foreign matter. In pure protein films, only 34 data points were found in an area equal to that examined by DIA, compared with >3000 data points collected in emulsion films. Therefore, the anisotropic regions measured by DIA can be assumed to be entirely lipid.

Light Microscopy. Under plane polarized light, anisotropic substances (crystals) can be differentiated from isotropic substances (whey protein matrix). Films viewed under polarized light appear in Figure 1. The WPI films containing myristic acid can be described as having a high concentration of minute needlelike par-

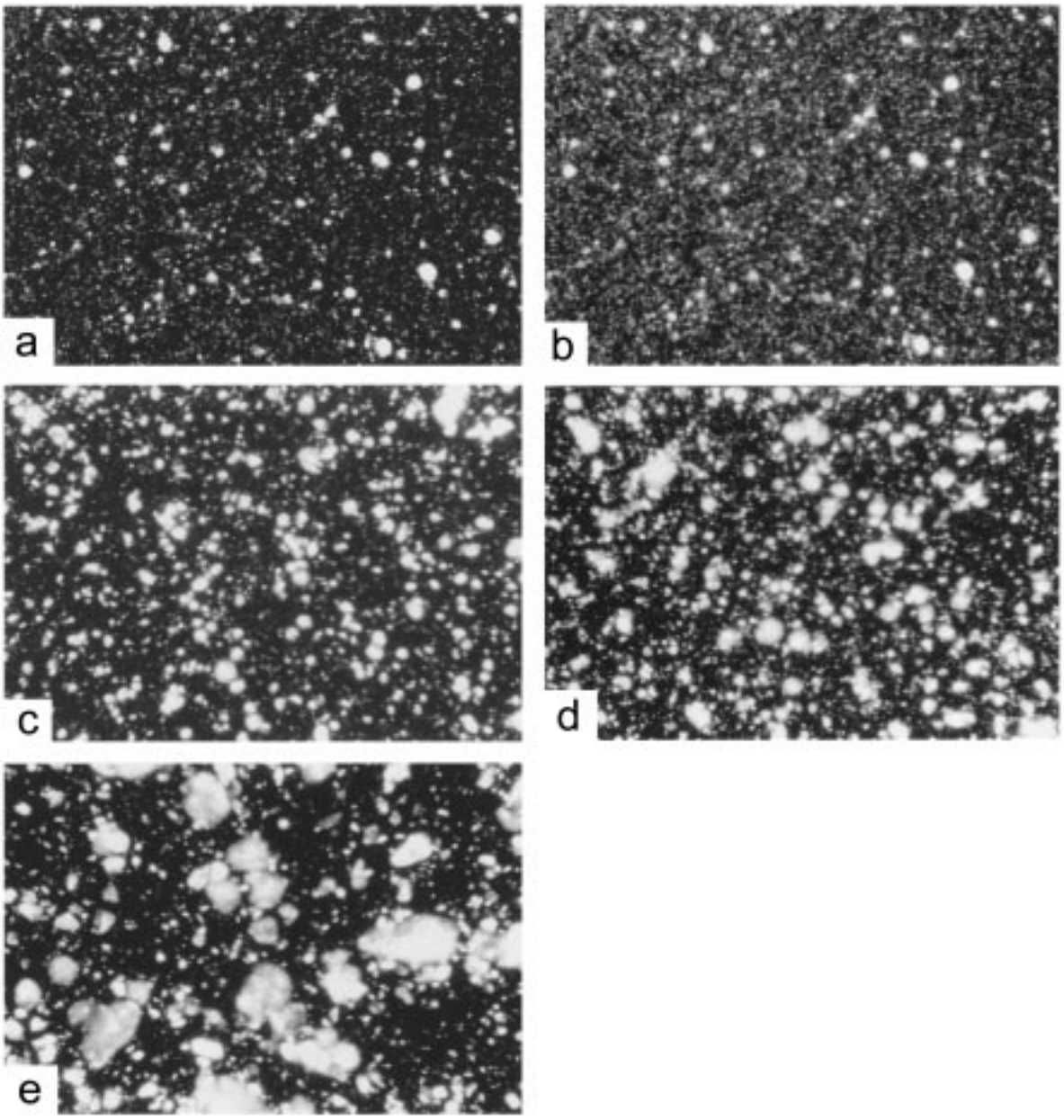

Figure 1. Emulsion films viewed by polarized light microscopy: (a) WPI−myristic acid; (b) WPI−palmitic acid; (c) WPI−stearic acid; (d) WPI−arachidic acid; (e) WPI−behenic acid.

ticles (Figure 2) with occasional somewhat larger crystals. Minute needlelike projections are indicative of β' crystals (deMan, 1982), the most common form for natural fats. Palmitic and stearic acid films were similar to each other, with a concentration of needlelike particles and an array of larger crystals. Arachidic and behenic acid films contained a small percentage of extremely large crystals and an array of different-sized crystals. These observations were confirmed in the EM experiments. Figure 2 shows SEM micrographs of representative crystals in myristic and behenic films. Although no one micrograph represents the entire microstructure, SEM can show more detail of distinctive objects witnessed in light microscopy.

DIA. Figure 3 shows a typical cumulative particle size distribution. As an example of the percentile comparison method, at the 75th percentile ~75% of the particles in the myristic acid formulation were $\leq 1\ \mu m^2$. Larger differences can be detected among the formulations at the higher percentiles (representing the very largest crystals). As FA chain length increases, the skew of the distribution increases, representing the presence of a few extremely large crystals.

The cumulative distributions show two shoulders at ~0.6 and ~0.8 μm^2, indicating a trimodal distribution. Multimodal distributions signify separate populations of lipid particles, differentiated by some unshared attribute. Bimodal distributions are sometimes indicative of clustering phenomena following homogenization (Langton and Hermansson, 1993); however, emulsion stability varied with chain length much more than these shoulders illustrate (Sherwin, 1998). More likely, the multiple peaks may represent the presence of different crystal types (deMan, 1982). Because the second shoulder (~0.8 μm^2) is less pronounced in the non-myristic acid films, which also had far fewer of the minute needlelike particles when viewed by both SEM and polarized light microscopy, sizes 0.8 μm^2 may be β' crystals. The first shoulder, which is found in all formulations, may simply reflect the limit of resolution

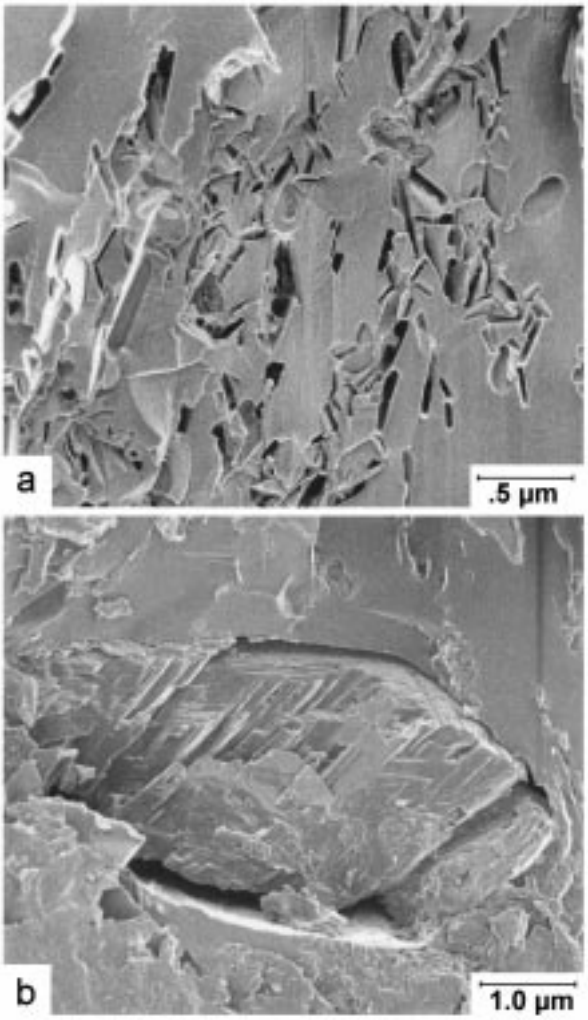

Figure 2. SEM micrographs of emulsion films: (a) WPI−myristic acid at 10000×; (b) WPI−behenic acid at 5000×.

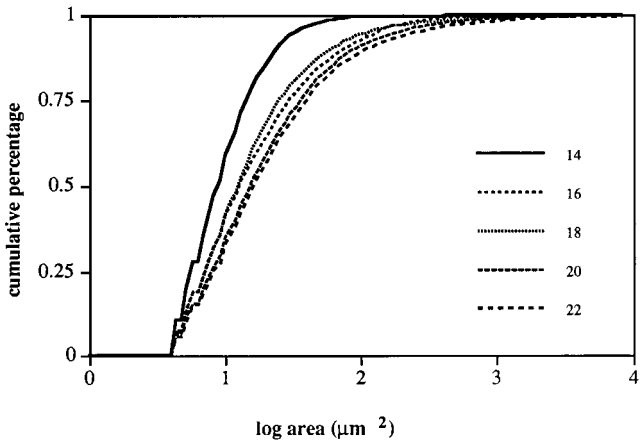

Figure 3. Typical dispersed phase particle size cumulative distribution of WPI−FA emulsion films.

of the digital camera. This particle size range accounts for only ~5% of the particles.

From the cumulative distribution, particle size can be seen to generally increase with FA chain length. Significant differences in formulation were found at a level of $\alpha = 0.05$ for all percentiles tested (Table 1). However, differences in both replication and duplication were found to be insignificant only at the 95th percentile and above, making a multiple comparison of means

Table 1. ANOVA of Cumulative Distribution at Four Percentiles

percentile	replicate *P* value (df = 4)	formulation *P* value (df = 4)
50	1.07×10^{-5}	4.62×10^{-8}
90	0.025	0.0009
95	0.069	0.0021
98	0.103	0.0024

Table 2. Mean Particle Sizes at 95th Percentile

formulation	particle size (μm)	
myristic	37	a
palmitic	144	ab
stearic	186	b
arachidic	187	b
behenic	338	c

Table 3. Mean Particle Sizes at 98th Percentile

formulation	particle size (μm)	
myristic	57	a
palmitic	269	ab
stearic	393	b
arachidic	425	bc
behenic	653	c

applicable. The 95th and 98th percentile points were averaged over all replications and Tukey's test was performed. Tables 2 and 3 show that particle size increased with FA chain length in three statistical groupings. Myristic and palmitic were lowest; palmitic, stearic, and arachidic were larger; and behenic was largest. Statistically, all films contained a similar profile of β' crystals and the smallest β crystals. Only among the largest β crystals were differences found.

Microstructural data can be used to determine the mode of inhibition, which the dispersed phase imparts on permeating water. Further research will explore the correlation among particle size, other microstructural parameters, and water vapor permeability.

Conclusions. Particle size increases with increasing chain length of FA in WP−FA emulsion films over the range of C_{14}−C_{22}. All formulations contain similar percentiles of small crystals, with very large globular crystals measured among arachidic and behenic acids. A bimodal distribution exists in dispersed phase particle size of WPI−FA emulsion films that represents two populations of FA crystals. These differences reflect differences in the manner in which crystals are formed during drying of the films.

ABBREVIATIONS USED

DIA, digital image analysis; EM, electron microscopy; FA, fatty acid; SEM, scanning electron microscopy; WPI, whey protein isolate; dWPI, denatured whey protein isolate; WVP, water vapor permeability; w/v, weight per unit volume.

LITERATURE CITED

Banerjee, R.; Chen, H. Functional Properties of Edible Films Using Whey Protein Concentrate. *J. Dairy Sci.* **1995**, *78*, 1673−1683.

Callegarin, F.; Quezada-Gallo, J. A.; Debeaufort, F.; Voilley, A. Lipids and Biopackaging. *J. Am. Oil Chem. Soc.* **1997**, *74*, 1183−1192.

Chen, H. Functional Properties and Applications of Edible Films Made of Milk Proteins. *J. Dairy Sci.* **1995**, *78*, 2563−2583.

Chen, H.; Banerjee, R.; Wu, J. R. *Proceedings of the Annual Conference of the American Society of Agricultural Engineers, Chicago, IL*; ASAE: St. Joseph, MI, 1993.

Debeaufort, F.; Voilley, A. Effect of Surfactants and Drying Rate on Barrier Properties of Emulsified Edible Films. *Int. J. Food Sci. Technol.* **1995**, *30*, 183−190.

Debeaufort, F.; Martin-Polo, M.; Voilley, A. Polarity Homogeneity and Structure Affect Water Vapor Permeability of Model Edible Films. *J. Food Sci.* **1993**, *58*, 426−434.

deMan, J. M. Microscopy in the Study of Fats and Emulsions. *Food Microsc.* **1982**, *1*, 209−222.

Fairley, P.; Krochta, J. M.; German, J. B. Interfacial Interactions in Edible Emulsion Films from Whey Protein Isolate. *Food Hydrocolloids* **1997**, *11*, 245−252.

Gejl-Hansen, F.; Flink, J. M. Freeze-Dried Carbohydrate Containing Oil-in-Water Emulsions: Microstructure and Fast Distribution. *J. Food Sci.* **1977**, *42*, 1049−1055.

Greener, I. K.; Fennema, O. Barrier Properties and Surface Characteristics of Edible, Bilayer Films. *J. Food Sci.* **1989**, *54*, 1393−1399.

Harrigan, K. A. Particle Size Analysis Using Automated Image Analysis. *Cereal Foods World* **1997**, *42*, 30−35.

Kamper, S. L.; Fennema, O. Water Vapor Permeability of Edible Bilayer Films. *J. Food Sci.* **1984**, *49*, 1478−1481.

Kester, J. J.; Fennema, O. An Edible Film of Lipids and Cellulose Ethers: Barrier Properties to Moisture Vapor Transmission and Structural Evaluation. *J. Food Sci.* **1989**, *54*, 1383−1389.

Koelsch, C. Edible Water Vapor Barriers: Properties and Promise. *Trends Food Sci. Technol.* **1994**, *51*, 76−81.

Krochta, J. M.; De Mulder-Johnston, C. Edible and Biodegradable Polymer Films: Challenges and Opportunities. *Food Technol.* **1997**, *51*, 61−74.

Langton, M.; Hermansson, A.-M. Image Analysis Determination of Particle Size Distribution. *Food Hydrocolloids* **1993**, *7*, 11−22.

McHugh, T. H.; Krochta, J. M. Dispersed Phase Particle Size Effects on Water Vapor Permeability of Whey Protein-Beeswax Edible Emulsion Films. *J. Food Process. Preserv.* **1994a**, *18*, 173−188.

McHugh, T. H.; Krochta, J. M. Water Vapor Permeability Properties of Edible Whey Protein−Lipid Emulsion Films. *J. Am. Oil Chem. Soc.* **1994b**, *71*, 307−312.

McHugh, T. H.; Aujard, J. F.; Krochta, J. M. Plasticized Whey Protein Edible Films: Water Vapor Permeability Properties. *J. Food Sci.* **1994**, *59*, 416−420.

Park, J. W.; Testin, R. F.; Vergano, P. J.; Park, H. J.; Weller, C. L. Fatty Acid Distribution and Its Effect on Oxygen Permeability in Laminated Edible Films. *J. Food Sci.* **1996**, *61*, 401−406.

Rosenberg, M.; Lee, S. L. Microstructure of Whey Protein/Anhydrous Milkfat Emulsions. *Food Struct.* **1993**, *12*, 267−274.

Shellhammer, T. H.; Krochta, J. M. Whey Protein Emulsion Film Performance as Affected by Lipid Type and Amount. *J. Food Sci.* **1997**, *62*, 390−394.

Sherwin, C. P. The Water Vapor Barrier Properties of Whey Protein-Fatty Acid Emulsion Films. Thesis, University of Minnesota, St. Paul, MN, 1998.

Stuchell, Y. M.; Krochta, J. M. Edible Coatings on Frozen King Salmon: Effect of Whey Protein Isolate and Aceetylated Monoglycerides on Moisture Loss and Lipid Oxidation. *J. Food Sci.* **1995**, *60*, 28−31.

Suttherawattananonda, M.; Fulcher, R. G.; Martin, F. B.; Bastian, E. D. Fluorescence Image Analysis of Process Cheese Manufactured with Trisodium Citrate. *J. Dairy Sci.* **1997**, *80*, 620−627.

Wong, D. W. S.; Gastineau, F. A.; Gregorski, K. S.; Tillin, S. J.; Pavlath, A. E. Chitosan−Lipid Films: Microstructure and Surface Energy. *J. Agric. Food Chem.* **1992**, *40*, 540−544.

Received for review March 23, 1998. Revised manuscript received August 26, 1998. Accepted August 26, 1998.

JF9802981