Microstructure of mixed gelatin-egg white gels: impact on rheology and application to microparticulation

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Microstructure of Mixed Gelatin–Egg White Gels: Impact on Rheology and Application to Microparticulation†

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The microstructure of mixed gelatin–egg white gels was examined by using both light and electron microscopy. Interpenetrating polymer network morphology was observed in mixed gels that were macroscopically single-phase systems. For two-phase gels, phase volumes were determined by optical image analysis and used to predict the composite shear modulus. Rheological properties were related to observable changes in the gel microstructure. For systems containing 5.6% egg white protein and greater than 5% gelatin, the dispersed egg white phase formed capsules of roughly 1–2 μm in diameter, with a dense outer coat and a less dense interior containing voids. From this, a method for the microparticulation of proteins based on aqueous phase partitioning was developed.

Introduction

Polymer blends have been defined as intimate mixtures of two kinds of polymers, with no covalent bonds between them (Manson and Sperling, 1976). After blending, polymers may be qualitatively considered incompatible, semicompatible, or compatible depending on whether two immiscible phases are formed, partial mixing takes place at the molecular level, or a single thermodynamically stable phase is formed. One of the earliest and simplest approaches to explain the mechanical behavior of synthetic polymer blends was the Takayanagi models, which predict the upper and lower bounds on the shear modulus (G) of a heterogeneous composite gel containing well-defined phase boundaries. For a system containing two polymers, X and Y, with individual shear moduli G_x and G_y, the isostrain model predicts the upper bound for the composite shear modulus as

$$G_c = \phi_x G_x + \phi_y G_y \quad (1)$$

where ϕ_x and ϕ_y are the volume fractions of X and Y, and the isostress model predicts the lower bound as

$$1/G_c = \phi_x/G_x + \phi_y/G_y \quad (2)$$

This requires the predicted value of G_c to lie between G_x and G_y.

As originally applied, the Takayanagi approach assumed pure, mutually insoluble components, whose individual rheological properties were independent of the macroscopic amounts present. Mixed biopolymer gels, however, differ markedly from binary blends of synthetic polymers in that a large volume fraction of solvent (usually water) is present in both the sol and the gel states (Clark et al., 1983). For mixed biopolymer gels, the way in which the solvent partitions itself between components X and Y will determine the appropriate values of G_x, G_y, ϕ_x, and ϕ_y to be inserted into eqs 1 and 2. Clark et al. (1983) have modified the original Takayanagi models, providing a quantitative means for predicting the bounds on G for mixed biopolymer gels by introducing an empirical parameter that accounts for solvent partitioning between the polymer constituents. The relative affinity parameter p, which was assumed to be independent of the overall polymer concentrations x and y, was given the functional form

$$\alpha = px/(px + y) \quad (3)$$

where α = fraction of solvent associated with the X phase. Clearly, $p < 1$ implies that Y is more solvent-attracting than X, the opposite being true for $p < 1$.

Assuming eq 3 to be valid, Clark et al. (1983) derived the following relationships:

$$C_x^{\text{eff}} = 100(px + y)/(100p + (1 - p)y) \quad (4)$$

$$C_y^{\text{eff}} = 100(px + y)/[100 - (1 - p)x] \quad (5)$$

$$\phi_x = x[100p + (1 - p)y]/100(px + y) \quad (6)$$

and

$$\phi_y = y[100 - (1 - p)x]/100(px + y) \quad (7)$$

For all x and y, provided p is known for the polymer pair and solvent in question and is constant over the concentration range studied, upper and lower limits on G_c can be calculated by using eqs 1 and 2 and the phase volume and effective concentration information supplied by eqs 4–7. To do this, ϕ_x and ϕ_y from eqs 6 and 7 are used directly in eqs 1 and 2, while C_x^{eff} and C_y^{eff} are used to determine the moduli G_x and G_y appropriate to the gel phases X and Y.

In a recent paper, Ziegler and Rizvi (1989) modeled the rheological behavior of mixed gelatin–egg white protein gels using the Takayanagi models as modified by Clark et al. (1983). Although complementary techniques were used, providing qualitative confirmation of the accuracy of the "relative affinity parameter", no direct measurement of the phase volumes, i.e., the solvent partitioning, was made. Furthermore, conclusions were drawn concerning the morphology of these gels solely on the basis of rheological data.

Microparticulation of protein into uniform, nearly spherical, and independent particles of 0.1–3.0 μm is the basis of methods to produce protein-based fat substitutes (Singer and Dunn, 1990). When hydrated, these particles have a smooth, creamy, emulsionlike organoleptic character (Singer et al., 1988; Singer and Dunn, 1990). Several recent patents have been issued covering methods for producing microparticulated protein and their use as fat substitutes (Kraft, Inc. 1989; Singer et al., 1988, 1989). Any technique used for microparticulation must produce

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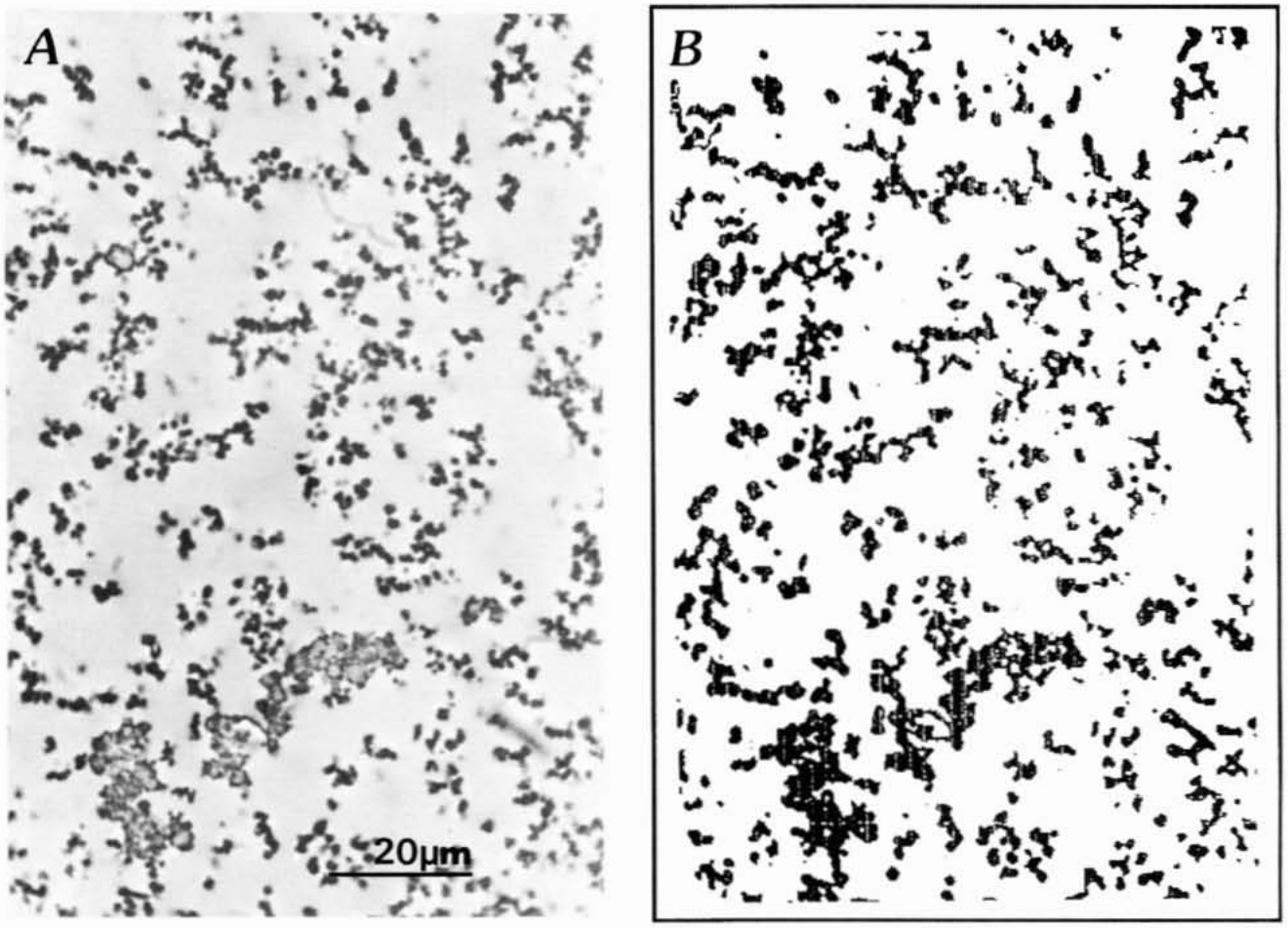

Figure 1. Light micrograph (A) and digitized image (B) for a gel containing 4.61% (w/w) type A gelatin, 5.60% (w/w) egg white protein in 0.6 M NaCl and 0.2 M $NaH_2PO_4 \cdot H_2O$ buffer at pH 6.0. Bar equals 20 μm.

aggregates of predictable size and size distribution and be reproducible if commercialization of the processes is to be realized.

This study provides direct measurement of gel-phase volumes using digital image processing and compares the results to those predicted empirically. Additionally, a method for the microparticulation of proteins is discussed.

Materials and Methods

Spray-dried egg white powder was obtained from Deb-El Foods Corp. (Elizabeth, NJ) or Henningsen Foods, Inc. (White Plains, NY). Type A, 275 Bloom gelatin, NaCl, NaN_3, and $NaH_2PO_4 \cdot H_2O$ were obtained from Fisher Scientific (Pittsburgh, PA). Microscopy supplies were obtained from Electron Microscopy Sciences (Ft. Washington, PA).

Mixed gelatin–egg white solutions were prepared in 0.6 M NaCl and 0.2 M $NaH_2PO_4 \cdot H_2O$ buffer at pH 6.0, following the procedure of Ziegler and Rizvi (1989). Gels were formed in no. 9800 Pyrex test tubes by heating 25 mL of mixed protein solutions at 80 °C for 1 h, followed by quenching in an ice/water slush and aging for 24 h at 5 °C.

One-millimeter cubes of gel were fixed in 3% glutaraldehyde in 0.15 M sodium cacodylate buffer (pH 7.1) for several days, then postfixed in 1% OsO_4 in 0.15 M sodium cacodylate buffer (pH 7.1) for 1 h. Gel pieces were then stained, en bloc, in 1% uranyl acetate in 0.1 M sodium acetate buffer for 1 h, dehydrated in a gradient series of acetone, and embedded in Spurr's low-viscosity medium. Thick sections (1 μm) were cut with a Sorvall MT-2 ultramicrotome, stained in 1% toluidine blue in 1% sodium borate, and observed with a Leitz Ortholux optical microscope. Thin sections (60 nm) were cut with an LKB Ultratome 3, stained with 3% uranyl acetate in 7.5% methanol, poststained in 2% lead citrate, and observed with a Hitachi Model 11E transmission electron microscope.

Table I. Phase Volume Fractions As Determined by Optical Image Processing

gelatin, g/100 g	egg white, g/100 g	n	ϕ_{egg}
2.87	5.60	2	0.2180 ± 0.0149
4.61	5.60	3	0.2134 ± 0.0175
7.66	5.60	3	0.2046 ± 0.0240

Microbeads of egg white protein were produced from solutions of 6.13% (w/w) egg white protein and 6.43% (w/w) type B gelatin in 0.6 M NaCl and 0.2 M $NaH_2PO_4 \cdot H_2O$ buffer at pH 6.0 by first mixing at 40 °C for 40 min, followed by quiescent heating at 80 °C for 1 h. Solutions were then washed twice by repeated dilution with 60 °C distilled water and centrifugation. Following centrifugation, the egg white microparticles were redispersed in distilled water by using a Branson Instruments Model S-75 sonifier. One milliliter of a dilute suspension of microparticles was filtered through a 0.2-μm polycarbonate filter (Gelman Sciences, Inc., Ann Arbor, MI). Samples on filters were fixed overnight at 4 °C in 3% glutaraldehyde in 0.15 M sodium cacodylate buffer at pH 7.1 and postfixed for 1 h in 1% OsO_4 in 0.15 M sodium cacodylate buffer at pH 7.1. Dehydration was accomplished through a gradient series of ethanol, followed by critical point drying in a Polaron E3000 critical point dryer using liquid CO_2 as the transitional fluid. Samples were

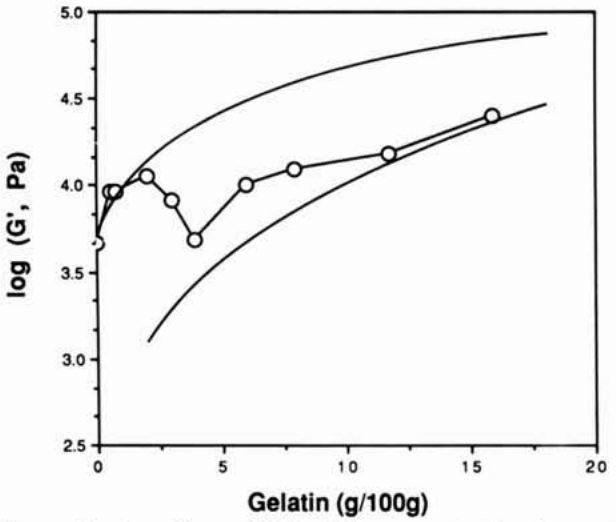

Figure 2. Log G' vs added gelatin concentration for a gel containing 6.04% (w/w) egg white protein in 0.6 M NaCl and 0.2 M $NaH_2PO_4 \cdot H_2O$ buffer at pH 6.0. Lines represent upper and lower bounds predicted from eqs 1, 2, and 4–7 by using a value of $p = 5$ [O: data from Ziegler and Rizvi (1989)].

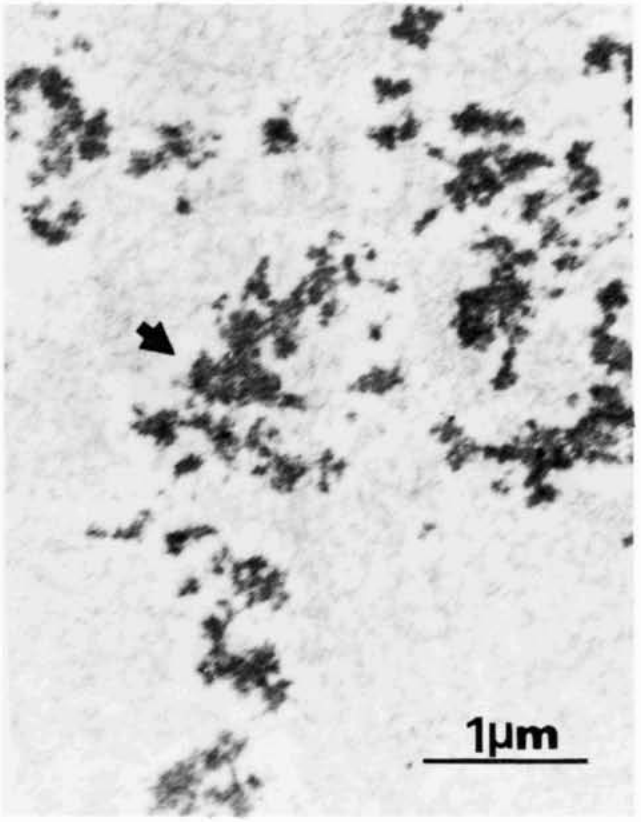

Figure 3. Transmission electron micrograph of a mixed gel containing 1.42% (w/w) type A gelatin (fine network) and 5.61% (w/w) egg white protein (coarse network) in 0.6 M NaCl and 0.2 M $NaH_2PO_4 \cdot H_2O$ buffer at pH 6.0. Gel was macroscopically single phase. Arrow indicates region devoid of protein. Bar equals 1 μm.

mounted on aluminum stubs, sputter-coated with approximately 28 nm of gold, and viewed on an International Scientific Instruments Model 60 scanning electron microscope at an accelerating voltage of 30 kV.

Digital image analysis was performed on a LeMont Scientific, Inc., OASYS optical analysis system. Image processing of micrographs was conducted to determine values of the area percent of the more dense phase. Digitized micrographs were considered representative two-dimensional sections of original three-dimensional samples containing randomly distributed, nearly spherical particles, and therefore, area fraction (area percent/100) was equated with volume fraction (Boatman, 1986). A Malvern Instruments "Mastersizer" high-resolution, laser diffraction particle size analyzer with a 45-mm focal length lens was used to determine particle size distributions of egg white particles suspended in distilled water.

The storage shear modulus, G', was determined at a frequency of 10 rad/s (1.590 Hz) at a maximum shear strain of 2.45% (1.96 mrad) using a Rheometrics thermal mechanical spectrometer equipped with a 0–100 g·cm transducer (Rheometrics, Inc., Piscataway, NJ). A parallel plate geometry with a 1-mm gap was used. For further experimental detail see Ziegler and Rizvi (1989).

Results and Discussion

Figure 1 is a representative light micrograph (A) and digitized image (B) of a mixed gel composed of 4.61% (w/w) type A gelatin and 5.60% (w/w) egg white protein. Selection of appropriate values for the limiting gray level was complicated by the fact that the phase boundaries were indistinct and hazy. Since both phases were protein, the staining procedure was nonselective. Therefore, the phases were distinguishable by stain density alone. Contrast between the phases was reduced by increasing thickness of the sections. This problem was less apparent at higher magnification. However, at higher magnification a smaller total area was being observed, possibly making the field under observation less representative of the entire sample. The size of the egg white phase "particles" observed in fixed and embedded samples (Figure 1) was consistent with that observed under the light microscope prior to fixation, dehydration, infiltration and sectioning.

By using the data in Table I, the relative affinity parameter, p, may be estimated from eq 7. The average value of p calculated from the data in Table I was between 5 and 6. This is slightly higher than the value of p of 2–3 determined empirically by Ziegler and Rizvi (1989). A value of $p < 1$ implies that the Y polymer (egg white) is more solvent-attracting than the X polymer (gelatin), the opposite being true for $p > 1$. Consequently, the boundaries calculated by the modified Takayanagi models change most noticeably for changes in p close to 1. Above $p = 3$ the boundaries are relatively insensitive to the value of p. Figure 2 is a comparison of experimentally determined values of the dynamic shear modulus (Ziegler and Rizvi, 1989) with the estimated bounds on G' calculated by using eqs 1, 2, and 4–7 with $p = 5$.

The nominal concentrations of gelatin and egg white protein in the gel depicted in Figure 1 were 4.61 and 5.60% (w/w), respectively. After phase separation and solvent partitioning, the true or effective concentrations of gelatin and egg white protein in their respective phases were approximately 5.8 and 27.8%.

The transition from the upper bound to the lower bound on G' (Figure 2), occurring between 2 and 4% added gelatin, was related to observable changes in the gel microstructure. Below 2% gelatin, mixed systems were macroscopically single phase, supported principally by the egg white network. Above 4% gelatin, gels were macroscopically two phase and supported by a continuous gelatin phase containing dispersed particles of egg white protein. Between 2 and 4% gelatin, varying proportions of large, continuous networks of egg white protein and small, dispersed particles of egg white were observed. Ziegler and Foegeding (1990) have recently reviewed the topic of mixed protein gels in detail.

Ziegler and Rizvi (1989) suggested that interpenetrating polymer networks (IPN's) were formed in mixed gelatin–egg white protein gels that were macroscopically single phase, but no direct confirmation was presented. For highly compatible polymers that form IPN's, both networks are continuous throughout the entire sample. Even with some degree of incompatibility, where partial phase separation may occur, the two components may remain intimately mixed, the dimensions of the phase domains being on the order of hundreds of angstroms and dependent on the degree of incompatibility (Manson and Sperling, 1976). IPN's have been found to exhibit a characteristic cellular structure, where the first network makes up the cell walls and the second the cell contents (Manson and Sperling, 1976). IPN's are possible even when the two networks are completely compatible, where thermodynamically only a single phase exists, if important differences such as the mechanism of gelation still remain that would allow for a gelation sequence to be established. Such is the case for gelatin and egg white protein.

Figure 3 is an electron micrograph of a mixed gelatin–egg white protein gel that was macroscopically single phase (no phase separation was observed upon extended centrifugation). Both the gelatin (fine, evenly dispersed) and egg white protein (dense, coarse) networks are visible. Even though the system was macroscopically single phase, some limited incompatibility is apparent, as evidenced by the regions devoid of protein at the interface between the networks (dark arrow). This also lends credibility to the assumption that the two networks are exclusive, i.e., they gel by vastly different mechanisms. The fine, evenly dispersed gelatin network is characteristic of transparent gels. The coarse network with greater fluctuations in protein density, larger pores, and thicker strands is characteristic of opaque, heat-set gels such as egg white.

Figure 4 is a transmission electron micrograph showing the structure of the egg white protein particles produced in two phase systems. Two types of particles were observed: small (≤0.5-μm diameter) spherical particles (light arrows) and larger (1–2-μm diameter) particles with a dense outer coat of protein and a less dense interior with regions devoid of protein (dark arrows). Voids seemed to be present only in particles that exceeded some critical size, roughly 0.5–1.0 μm. This interpretation is somewhat tentative, since the smaller solid particles may have been an artifact created by sectioning through the solid portion of a larger protein capsule. However, both scanning electron microscopy and particle size analyses confirmed the presence of particles with diameters below 0.5 μm, and very few particles of this diameter or less showed internal voids. Aggregation of particles distorted the shape somewhat and led to the formation of "grapelike" clusters. Similar structures have been observed in acid-heat-induced milk gels (Harwalkar and Kalab, 1988).

Under the appropriate conditions, aqueous phase partitioning of egg white and gelatin leads to the formation of a two-phase system with a continuous phase of gelatin–water and a dispersed phase of egg white–water that resembles a typical oil-in-water emulsion. Phase separation in binary polymer mixtures may result from either nucleation and growth (from the metastable region) or from spinodal decomposition (from the unstable regions) (Bates, 1991). Minimization of interfacial tension through reduction of interfacial area is the driving force behind coarsening. Because of the low diffusivity and high viscosity of polymers, coarsening is often very slow (Bates, 1991). For polymers that gel, this allows time for the dispersed phase to be set into solid particles while they are still quite small.

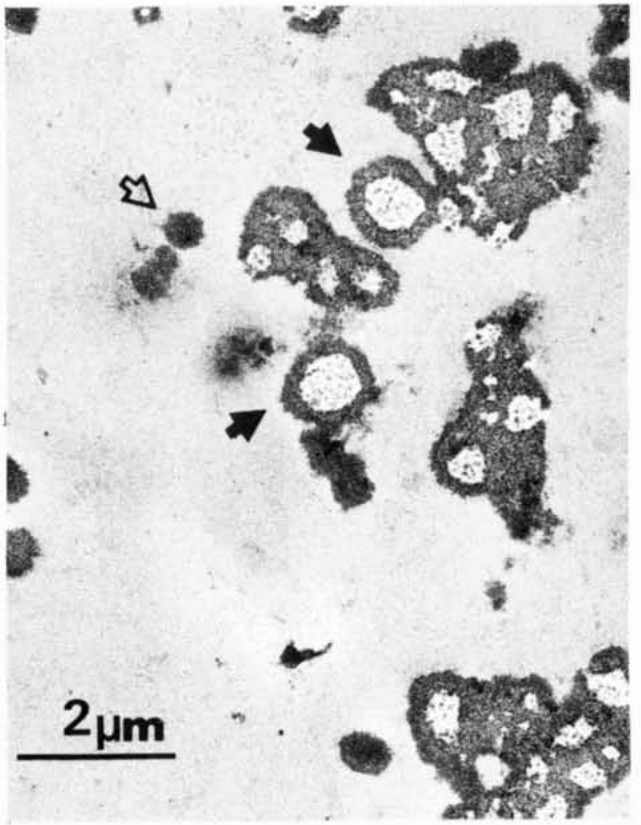

Figure 4. Transmission electron micrograph of egg white protein microparticles. Dark arrows, egg white capsules; light arrows, solid egg white particles. Bar equals 2 μm.

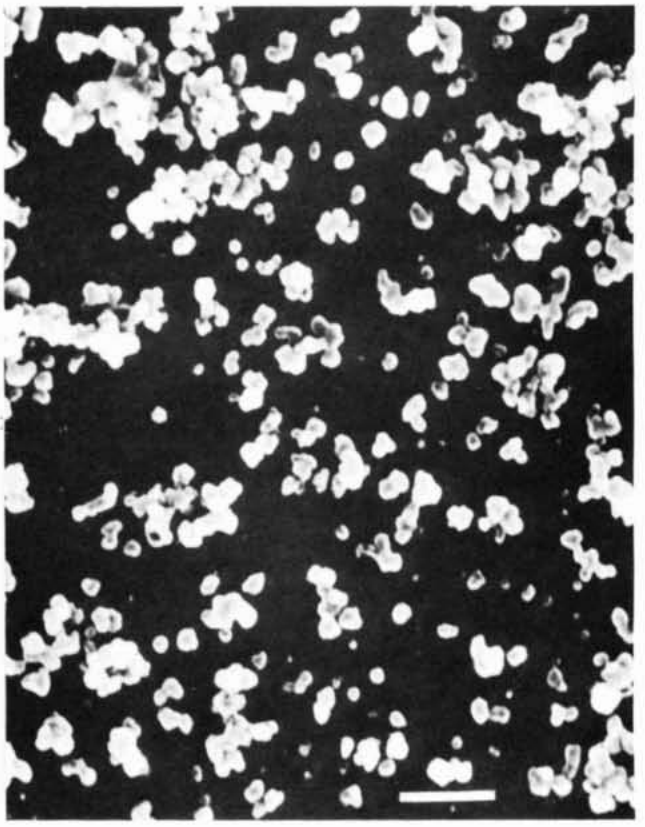

Figure 5. Scanning electron micrograph of microparticles of egg white protein. Bar equals 10 μm.

Under shear, the size of the egg white phase domain can be reduced, and on heating, microparticles of egg white

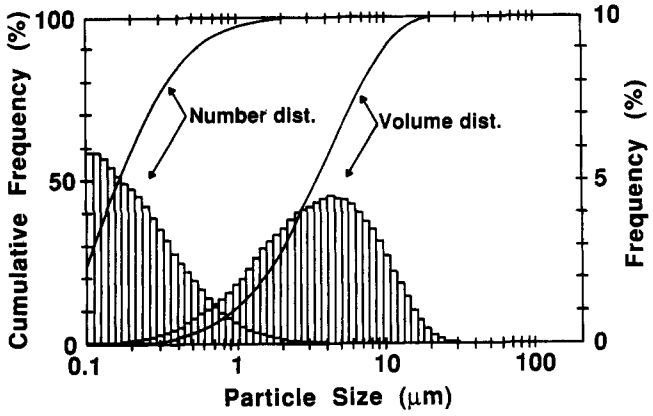

Figure 6. Particle size distributions (number and volume) of egg white microparticles.

formed. Figure 5 shows the surface characteristics of egg white microparticles produced by mixing, followed by heating/setting, centrifugation, and finally drying. Particle size and the degree of agglomeration were controlled by varying the shear. Dispersion before, during, or after heating produced differing effects. The particle size distribution of egg white microparticles prior to drying, and produced under low shear conditions, are shown in Figure 6. Simple fragmentation of heat-set gel networks, while producing "microparticulated" protein, does not result in the uniform, spherical particles that may be necessary to simulate the mouthfeel of fat.

Conclusions

Rheological behavior of mixed gelatin–egg white gels was related to observable changes in gel microstructure. The composite shear modulus was accurately modeled by using the Takayanagi approach coupled with digital image analysis to determine phase volumes. Interpenetrating network morphology was observed in single-phase gels, suggesting exclusivity in network formation. Microparticles of egg white protein approximately 1–2 μm in diameter were produced by a relatively simple process.

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